FILE 'REGISTRY' ENTERED AT 11:05:39 ON 17 AUG 2004

=> S CELLOBIOHYDROLASE/CN
L1 1 CELLOBIOHYDROLASE/CN

=> D

L1 ANSWER 1 OF 1 REGISTRY COPYRIGHT 2004 ACS on STN
RN 37329-65-0 REGISTRY
CN Cellobiohydrolase, exo- (9CI) (CA INDEX NAME)
OTHER NAMES:
CN β-1,4-Cellobiosidase
CN β-1,4-Glucan cellobiohydrolase
CN β-1,4-Glucan cellobiosylhydrolase
CN β-D-Cellobiopyranosidase
CN β-D-Cellobiosidase
CN 1,4-β-D-Glucan cellobiohydrolase
CN 1,4-β-Glucan cellobiohydrolase
CN 1,4-β-Glucan cellobiosidase
CN Avicelase II
CN C1 Cellulase
CN **Cellobiohydrolase**
CN Cellobiohydrolase I
CN Cellobiohydrolase II
CN Cellobiosidase
CN Cellobiosidase, 1,4-β-glucan
CN Cellulase, C1
CN Cellulose 1,4-β-cellobiosidase
CN E.C. 3.2.1.91
CN Exo-β-1,4-glucan cellobiohydrolase
CN Exo-1,4-β-D-cellobiohydrolase
CN Exocellobiohydrolase
CN Spezyme CP
DR 187112-53-4
MF Unspecified
CI MAN
LC STN Files: AGRICOLA, ANABSTR, BIOBUSINESS, BIOSIS, BIOTECHNO, CA, CAPLUS, CASREACT, CHEMLIST, CIN, EMBASE, PIRA, PROMT, TOXCENTER, USPAT2, USPATFULL
Other Sources: EINECS**
(**Enter CHEMLIST File for up-to-date regulatory information)
DT.CA CAplus document type: Conference; Dissertation; Journal; Patent; Report
RL.P Roles from patents: ANST (Analytical study); BIOL (Biological study); MSC (Miscellaneous); OCCU (Occurrence); PREP (Preparation); PROC (Process); PRP (Properties); USES (Uses)
RLD.P Roles for non-specific derivatives from patents: ANST (Analytical study); BIOL (Biological study); PREP (Preparation); PRP (Properties); USES (Uses)
RL.NP Roles from non-patents: ANST (Analytical study); BIOL (Biological study); FORM (Formation, nonpreparative); MSC (Miscellaneous); OCCU (Occurrence); PREP (Preparation); PROC (Process); PRP (Properties); RACT (Reactant or reagent); USES (Uses)
RLD.NP Roles for non-specific derivatives from non-patents: ANST (Analytical study); BIOL (Biological study); MSC (Miscellaneous); PREP (Preparation); PROC (Process); PRP (Properties); USES (Uses)

*** STRUCTURE DIAGRAM IS NOT AVAILABLE ***
1277 REFERENCES IN FILE CA (1907 TO DATE)

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             47 REFERENCES TO NON-SPECIFIC DERIVATIVES IN FILE CA
           1278 REFERENCES IN FILE CAPLUS (1907 TO DATE)

FILE 'CAPLUS' ENTERED AT 11:06:07 ON 17 AUG 2004

=> S CELLOBIOHYDROLASE;S L1;S L1,L2
          1120 CELLOBIOHYDROLASE
           265 CELLOBIOHYDROLASES
L2        1191 CELLOBIOHYDROLASE
                 (CELLOBIOHYDROLASE OR CELLOBIOHYDROLASES)


L3        1278 L1


          1278 L1
L4        1556 (L1 OR L2)

=> S THERMAL;S STABILITY;S L5(3A)L6
        943295 THERMAL
            66 THERMALS
L5      943324 THERMAL
                 (THERMAL OR THERMALS)


        586896 STABILITY
         22689 STABILITIES
L6      597962 STABILITY
                 (STABILITY OR STABILITIES)


L7       86547 L5(3A)L6

=> S LINKER;S DOMAIN;S CATALYTIC;S CELLULOSE BINDING
         15778 LINKER
          3720 LINKERS
L8       17911 LINKER
                 (LINKER OR LINKERS)


        229333 DOMAIN
        121660 DOMAINS
L9      289552 DOMAIN
                 (DOMAIN OR DOMAINS)


        369332 CATALYTIC
            26 CATALYTICS
L10     369341 CATALYTIC
                 (CATALYTIC OR CATALYTICS)


        317676 CELLULOSE
          4103 CELLULOSES
        318141 CELLULOSE
                 (CELLULOSE OR CELLULOSES)
        828724 BINDING
          1831 BINDINGS
        829238 BINDING
                 (BINDING OR BINDINGS)
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L11        1070 CELLULOSE BINDING
                (CELLULOSE(W)BINDING)

=> S L10(W)L9;S L11(W)L9;S GLYCOSYLATION;S AMINO ACID
L12        6607 L10(W)L9


L13         691 L11(W)L9


          29584 GLYCOSYLATION
            457 GLYCOSYLATIONS
L14       29706 GLYCOSYLATION
                (GLYCOSYLATION OR GLYCOSYLATIONS)


         989169 AMINO
             42 AMINOS
         989186 AMINO
                (AMINO OR AMINOS)
        3856116 ACID
        1442277 ACIDS
        4325218 ACID
                (ACID OR ACIDS)
L15      623817 AMINO ACID
                (AMINO(W)ACID)

=> S L4 AND L8
L16          46 L4 AND L8

=> S L4(6A)L8
L17           6 L4(6A)L8

=> S L4(12A)L8
L18          10 L4(12A)L8

=> S L12 AND L13 AND L8
L19          62 L12 AND L13 AND L8

=> S L19 AND L18
L20           2 L19 AND L18

=> S L19 NOT L20
L21          60 L19 NOT L20

=> S L18 NOT L20
L22           8 L18 NOT L20

=> D L22 1-8 CBIB ABS;D L21 1-60 TI

L22  ANSWER 1 OF 8  CAPLUS  COPYRIGHT 2004 ACS on STN
2002:145756  Document No. 136:337665  Expression and processing of a major
     xylanase (XYN2) from the thermophilic fungus Humicola grisea var.
     thermoidea in Trichoderma reesei.  De Faria, F. P.; Te'o, V. S. J.;
     Bergguist, P. L.; Azevedo, M. O.; Nevalainen, K. M. H. (FAENQUIL,
     Departamento de Biotecnologia, Lorena, Brazil).  Letters in Applied
     Microbiology, 34(2), 119-123 (English) 2002.  CODEN: LAMIE7.  ISSN:
     0266-8254.  Publisher: Blackwell Publishing Ltd..
AB   Aims: To express a gene encoding a heterologous fungal xylanase in Trichoderma
     reesei.  Methods and Results: Humicola grisea xylanase 2 (xyn2) cDNA was
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expressed in Trichoderma reesei under the main cellobiohydrolase I (cbh1) promoter (i) as a fusion to the **cellobiohydrolase** I (CBHI) secretion signal and (ii) the mature CBHI core-**linker**. The recombinant xylanase (HXYN2) was secreted into the cultivation medium and processed in a similar fashion to the endogenous T. reesei xylanases, resulting in an active enzyme. Conclusions, Significance and Impact of the Study: HXYN2 was successfully processed in T. reesei. Composition of the culture medium affected the HXYN2 yields, favoring Avicellactose as a carbon source. Best yields (about 0.5 g l-1) in shake flask cultivations were obtained from a transformant where xyn2 was fused directly to the CBHI secretion signal.

L22 ANSWER 2 OF 8 CAPLUS COPYRIGHT 2004 ACS on STN

2000:301991 Document No. 133:262111 Cloning, characterization and chromosomal location of three genes encoding host-cell-wall-degrading enzymes in Leptosphaeria maculans, a fungal pathogen of Brassica spp.. Sexton, A. C.; Paulsen, M.; Woestemeyer, J.; Howlett, B. J. (School of Botany, The University of Melbourne, Parkville, Australia). Gene, 248(1-2), 89-97 (English) 2000. CODEN: GENED6. ISSN: 0378-1119. Publisher: Elsevier Science B.V..

AB The ascomycete, Leptosphaeria maculans, causes blackleg disease of oilseed Brassica spp. such as canola (Brassica napus). We have cloned a gene encoding endopolygalacturonase, pg1, and two genes encoding cellulases, cel1 and cel2, in L. maculans. These genes are not clustered in the genome, as they are located on different chromosomes. The deduced amino acid sequences of all three genes predict an N-terminal signal sequence, as is common for secreted fungal enzymes that degrade plant cell walls. The endopolygalacturonase encoded by pg1 shows the highest similarity (54% amino acid identity) to endopolygalacturonase 4 from Botrytis cinerea. Both cel1 and cel2 appear to encode **cellobiohydrolase**, and neither gene encodes a recognizable cellulose-binding domain or **linker** region. Transcription of pg1 is induced in cultures containing 1% polygalacturonic acid or pectin, and cel1 is induced in 1% cellulose or CM-cellulose, as shown by Northern anal. Glucose represses the induction of cel1 caused by cellulose and CM-cellulose, but does affect transcription of pg1. Transcription of cel2 (but not cel1 or pg1) is detectable during infection of B. napus and B. juncea cotyledons and leaves using reverse transcription-PCR.

L22 ANSWER 3 OF 8 CAPLUS COPYRIGHT 2004 ACS on STN

1999:428887 Document No. 131:198290 Expression and characterization of bispecific single-chain Fv fragments produced in transgenic plants. Fischer, Rainer; Schumann, Detlef; Zimmermann, Sabine; Drossard, Jurgen; Sack, Markus; Schillberg, Stefan (Fraunhofer Abteilung fur Molekulare Biotechnologie, IUCT, Schmallenberg, Germany). European Journal of Biochemistry, 262(3), 810-816 (English) 1999. CODEN: EJBCAI. ISSN: 0014-2956. Publisher: Blackwell Science Ltd..

AB The authors describe the expression of the bispecific antibody biscFv2429 in transgenic suspension culture cells and tobacco plants, biscFv2429 consists of two single-chain antibodies, scFv24 and scFv29, connected by the Trichoderma reesi **cellobiohydrolase** I **linker**, biscFv2429 binds two epitopes of tobacco mosaic virus (TMV): the scFv24 domain recognizes neotopes of intact virions, and the scFv29 domain recognizes a cryptotope of the TMV coat protein monomer. BiscFv2429 was functionally expressed either in the cytosol (biscFv2429-cyt) or targeted to the apoplast using a murine leader peptide sequence (biscFv2429-apoplast). A third construct contained the C-terminal KDEL sequence for retention in the ER (biscFv2429-KDEL). Levels of cytoplasmic biscFv2429 expression levels were low. The highest levels of antibody expression were for apoplast-targeted biscFv2429-apoplast and ER-retained biscFv2429-KDEL that reached a maximum expression level of 1.65% total soluble

protein in transgenic plants. Plant-expressed biscFv2429 retained both epitope specificities, and bispecificity and bivalency were confirmed by ELISA and surface plasmon resonance anal. This study establishes plant cells as an expression system for bispecific single-chain antibodies for use in medical and biol. applications.

L22 ANSWER 4 OF 8 CAPLUS COPYRIGHT 2004 ACS on STN
1996:541940 Document No. 125:215577 Characterization of a double cellulose-binding domain. Synergistic high affinity binding to crystalline cellulose. Linder, Markus; Salovuori, Irma; Ruohonen, Laura; Teeri, Tuula T. (VTT, Biotechnol. Food Res., FIN-02044, Finland). Journal of Biological Chemistry, 271(35), 21268-21272 (English) 1996. CODEN: JBCHA3. ISSN: 0021-9258. Publisher: American Society for Biochemistry and Molecular Biology.

AB Most cellulose-degrading enzymes have a two-domain structure that consists of a catalytic and a cellulose-binding domain (CBD) connected by a linker region. The linkage and the interactions of the two domains represent one of the key questions for the understanding of the function of these enzymes. The CBDs of fungal cellulases are small peptides folding into a rigid, disulfide-stabilized structure that has a distinct cellulose binding face. Here we describe properties of a recombinant double CBD, constructed by fusing the CBDs of two Trichoderma reesei **cellobiohydrolases** via a **linker** peptide similar to the natural cellulase **linkers**. After expression in Escherichia coli, the protein was purified from the culture medium by reversed phase chromatog. and the individual domains obtained by trypsin digestion. Binding of the double CBD and its single CBD components was investigated on different types of cellulose substrates as well as chitin. Under saturating conditions, nearly 20 μmol/g of the double CBD was bound onto microcryst. cellulose. The double CBD exhibited much higher affinity on cellulose than either of the single CBDs, indicating an interplay between the two components. A two-step model is proposed to explain the binding behavior of the double CBD. A similar interplay between the domains in the native enzyme is suggested for its binding to cellulase.

L22 ANSWER 5 OF 8 CAPLUS COPYRIGHT 2004 ACS on STN
1995:742015 Document No. 123:247860 Multiple roles of the cellulase CBHI in enhancing production of fusion antibodies by the filamentous fungus Trichoderma reesei. Nyyssonen, Eini; Keranen, Sirkka (VTT Biotechnology and Food Research, FIN-02044, Finland). Current Genetics, 28(1), 71-9 (English) 1995. CODEN: CUGED5. ISSN: 0172-8083. Publisher: Springer.

AB The production of Fab antibody fragments in Trichoderma reesei can be increased over 50-fold by fusing the core-**linker** region of the T. reesei cellulase CBHI (**cellobiohydrolase** I) to the heavy Fd chain (Nyyssonen et al. 1993). This beneficial role of CBHI in antibody production has now been studied further by comparisons of T. reesei strains producing the light chain only, Fab or CBHI-Fab all of which exhibited identical light chain integration. The N-terminal fusion of CBHI to the heavy Fd chain not only aided secretion, as expected, but also increased the level of mRNA encoding the CBHI-heavy Fd chain, either by stabilizing the messenger or by enhancing transcription. The CBHI part appeared to facilitate secretion at least by aiding the passage through the endoplasmic reticulum, since processing of the signal peptide of the antibody chains seemed to be most efficient in the strain producing CBHI-Fab in contrast to the strains producing light chain or Fab fragment. Interestingly, CBHI core-linker protein, originating from the CBHI-heavy Fd chain, was found in large amts. in the culture medium. The cleavage resulting in this tailless CBHI occurred inside the cell. This suggests that, by omitting the heterologous tail, the secretion of the

resulting CBHI core-linker protein is enhanced to a level comparable with secretion of the extracellular T. reesei proteins.

L22 ANSWER 6 OF 8 CAPLUS COPYRIGHT 2004 ACS on STN
1993:554801 Document No. 119:154801 Role of the interdomain **linker** peptide of Trichoderma reesei **cellobiohydrolase** I in its interaction with crystalline cellulose. Srisodsuk, Malee; Reinikainen, Tapani; Penttila, Merja; Teeri, Tuula T. (Biotech. Lab., VTT, Espoo, SF-02151, Finland). Journal of Biological Chemistry, 268(28), 20756-61 (English) 1993. CODEN: JBCHA3. ISSN: 0021-9258.

AB Cellobiohydrolase I (CBH I), the major component of Trichoderma reesei cellulolytic system, is comprised of a catalytic core domain joined to a cellulose binding-domain (CBD) by an extended O-glycosylated interdomain linker peptide. Two internal deletions were introduced to the linker in order to investigate its function particularly in the hydrolysis of crystalline cellulose. Deletion of the first one-third of the linker, including a putative hinge region, reduces the binding capacity of CBH I in high enzyme coverage but does not affect its enzymic activity on crystalline cellulose. The longer deletion removing practically all of the linker dramatically reduces the rate of crystalline cellulose degradation even though the enzyme still binds to the substrate. Thus, sufficient spatial separation of the two domains is required for efficient function of CBH I. It is evident that the presence of a functional CBD is increasingly important for CBH I toward higher enzyme to cellulose ratios. Apparently, the putative hinge removed by the first deletion facilitates CBD-driven binding and dense packing of the wild type enzyme on the cellulose surface.

L22 ANSWER 7 OF 8 CAPLUS COPYRIGHT 2004 ACS on STN
1993:535292 Document No. 119:135292 Secretion of thermophilic bacterial cellobiohydrolase in Saccharomyces cerevisiae. Uozumi, Nobuyuki; Hayashi, Akihiro; Ito, Takaomi; Patthra, Arunwanich; Yamashita, Ichiro; Iijima, Shinji; Kobayashi, Takeshi (Fac. Eng., Nagoya Univ., 464-01, Japan). Journal of Fermentation and Bioengineering, 75(6), 399-404 (English) 1993. CODEN: JFBIEX. ISSN: 0922-338X.

AB The partial DNA sequence corresponding to the N-terminal amino acid sequence of cellobiohydrolase derived from a thermophilic anaerobe NA10 was determined The cellobiohydrolase gene fused to the secretion signal (signal peptide and T-S region) from Saccharomyces diastaticus was expressed in an ethanologenic yeast, S. cerevisiae YIY345, under control of the glucoamylase promoter. The recombinant yeast produced cellobiohydrolase; .apprx.40% of the total cellobiohydrolase activity was detected in the medium, and the remaining cellobiohydrolase was localized in the intracellular fraction. An anal. of the N-terminal amino acid sequence of the main intracellular cellobiohydrolase revealed that the signal peptide and T-S region were removed proteolytically. Alteration of the amino acid residues at the cleavage site by insertion of a BglII **linker** led to an .apprx.3.5-fold increase in the total **cellobiohydrolase** production, but did not affect the efficiency of secretion into the medium. Cellobiohydrolase production was not repressed in the presence of glucose. The recombinant yeast hydrolyzed CM-cellulose in the medium. The results suggest the possibility of the direct bioconversion of cellulose to EtOH by the recombinant yeast.

L22 ANSWER 8 OF 8 CAPLUS COPYRIGHT 2004 ACS on STN
1991:653792 Document No. 115:253792 An active single-chain antibody containing a cellulase linker domain is secreted by Escherichia coli. Takkinen, Kristiina; Laukkanen, Marja Leena; Sizmann, Dorothea; Alfthan, Kaija; Immonen, Tiina; Vanne, Liisa; Kaartinen, Matti; Knowles, Jonathan

K. C.; Teeri, Tuula T. (VTT Biotech. Lab., Espoo, SF-02151, Finland). Protein Engineering, 4(7), 837-41 (English) 1991. CODEN: PRENE9. ISSN: 0269-2139.

AB Single-chain antibodies consist of the variable, antigen-binding domains of antibodies joined to a continuous polypeptide by genetically engineered peptide linkers. The flexible interdomain linker region of a fungal cellulase was used to link together the variable domains of an anti-2-phenyloxazolone IgG1 and it is shown here that the resulting single-chain antibody is efficiently secreted and released to the culture medium of E. coli. The yield of affinity-purified single-chain antibody is 1-2 mg/L of culture medium and its affinity and stability are comparable to those of the corresponding native IgG.

=> D L21 7,8,12,16-19,21,23,27,29,31,33,35-37,39,41,44-48,50,54-60 CBIB ABS

L21 ANSWER 7 OF 60 CAPLUS COPYRIGHT 2004 ACS on STN

2002:186509 Characterisation and expression in-planta of a fungal **cellulose-binding domain**. Quentin, Michael; Derksen, Jan; DeJong, Ed; Mariani, Celestina; VanderValk, Henry (Department of Fibre & Paper Technology, ATO BV, Wageningen, 6700 AA, Neth.). Abstracts of Papers, 223rd ACS National Meeting, Orlando, FL, United States, April 7-11, 2002, CELL-099. American Chemical Society: Washington, D. C. (English) 2002. CODEN: 69CKQP.

AB Fiber surface structure plays a key role in fiber properties, and their chemical composition depends on the raw material and on the production process. Targetted enzymic modification of the fiber surface may lead to improved tech. qualities and processibility of the fibers. In addition, modification of the biosynthesis and assembly of cell walls of fiber-yielding plants is of great interest in optimizing requested fibers properties. Most cellulases of microbial origine have a three-domain structure consisting of a **catalytic domain**, a **cellulose- binding domain** (CBD), separated by a distinct **linker** region. The CBD seems to be responsible for targetting and for facilitating the activity of the **catalytic domain** on insol. cellulosic substrate. CBDs are stucturally and functionally independent from the **catalytic domain**; and as they can be used to construct fusion proteins without affecting the biol. activity of the hybrid (1), they represent interesting tools for fiber surface modification. Bacterial CBDs have also been shown to enhance plant development and cellulose yield when expressed in plants (2), and could be used to modify fibers quality in-planta. A **cellulose- binding domain** and a serine- and threonine-rich **linker** peptide were cloned from the fungi Aspergillus japonicus and Aspergillus aculeatus. A fusion protein GST-CBD, in which the glutathione S-transferase was linked to the peptide **linker** and to the **cellulose-binding domain** at its C-terminus, was expressed in Escherichia coli. Renaturated GST-CBD, recovered from inclusion bodies and purified on Glutathione Sepharose, adsorbed to both bacterial microcryst. cellulose and CM-cellulose. Deletion of the **linker** peptide affected the ability of the hybrid protein to adsorb to cellulose, and made it more sensitive to protease digestion. The sequences coding for this fungal **cellulose-binding domain** was introduced in Arabidopsis thaliana via Agrobacterium thumefaciens. Plants expressing the **cellulose-binding domain** were shown to be affected in their development. Ong et. al. 1989. Trends in Biotechnol. Vol.7. pp.239-243. Shpigel et. al. 1998. Plant Physiol. Vol.117. pp.1185-1194.

L21 ANSWER 8 OF 60 CAPLUS COPYRIGHT 2004 ACS on STN

2002:186423 Construction and characterization of a Coprinus cinereus peroxidase variant fused with a **cellulose-binding domain**. Xu, Feng; Jones, Aubrey; Conrad, Lars S.; Cherry, Joel R.

(Novozymes Biotech, Davis, CA, 95616, USA). Abstracts of Papers, 223rd ACS National Meeting, Orlando, FL, United States, April 7-11, 2002, CELL-013. American Chemical Society: Washington, D. C. (English) 2002. CODEN: 69CKQP.

AB The **catalytic domain** sequence of the Humicola insolens Cel45 endo-1,4-glucanase was genetically replaced by the encoding sequence of Coprinus cinereus peroxidase, leading to a hybrid protein comprising the **cellulose-binding-domain, linker** , and peroxidase. The hybrid protein was expressed in Aspergillus oryzae, purified, and found to retain 80% of the peroxidase activity and possess 200% more affinity toward avicel cellulose.

L21 ANSWER 12 OF 60 CAPLUS COPYRIGHT 2004 ACS on STN

2001:430751 Document No. 135:104253 Do domain interactions of glycosyl hydrolases from Clostridium thermocellum contribute to protein thermostability?. Kataeva, Irina A.; Blum, David L.; Li, Xin-Liang; Ljungdahl, Lars G. (Center for Biological Resources Recovery and Department of Biochemistry and Molecular Biology, University of Georgia, Athens, GA, 30602-7229, USA). Protein Engineering, 14(3), 167-172 (English) 2001. CODEN: PRENE9. ISSN: 0269-2139. Publisher: Oxford University Press.

AB Cellulolytic and hemicellulolytic enzymes usually have a domain composition The mutual influence of a **cellulose-binding domain** (CBD) and a **catalytic domain** was investigated with cellobiohydrolase CelK and xylanase XynZ from C. thermocellum. CelK is composed of an N-terminal family IV CBD (CBDIVCelK), a family 9 glycosyl hydrolase domain (Gh9CelK) and a dockerin domain (DD). CelK without the DD, (CBDIV-Gh9)CelK and CBDIVCelK bound cellulose. The thermostability of (CBDIV-Gh9)CelK was significantly higher than that of CBDIVCelK and Gh9CelK. The temperature optima of (CBDIV-Gh9)CelK and Gh9CelK were 65 and 45°, resp. XynZ consists of an N-terminal feruloyl esterase domain (FAEXynZ), a **linker** (L), a family VI CBD (CBDVIXynZ), a DD, and a xylanase domain. FAEXynZ and (FAE-L-CBDVI)XynZ, used in the present study did not bind cellulose, but both were highly thermostable. Replacement of CBDVIXynZ with CBDIVCelK resulted in chimeras with feruloyl esterase activity and the ability to bind cellulose. CBDIVCelK-FAEXynZ bound cellulose with parameters similar to that of (CBDIV-Gh9)CelK. (FAE-L)XynZ-CBDIVCelK and FAEXynZ-CBDIVCelK had lower relative affinities and binding capacities than those of (CBDIV-Gh9)CelK. The 3 chimeras were much less thermostable than FAEXynZ and (FAE-L-CBDVI)XynZ. The results indicated that domains of glycosyl hydrolases are not randomly combined and that domain interactions affect the properties of these domain-structured enzymes.

L21 ANSWER 16 OF 60 CAPLUS COPYRIGHT 2004 ACS on STN

2000:708675 Document No. 135:30657 Isolation of β-1,4-endoglucanase genes from Globodera tabacum and their expression during parasitism. Goellner, M.; Smant, G.; De Boer, J. M.; Baum, T. J.; Davis, E. L. (Department of Plant Pathology, North Carolina State University, Raleigh, NC, 27695, USA). Journal of Nematology, 32(2), 154-165 (English) 2000. CODEN: JONEB5. ISSN: 0022-300X. Publisher: Society of Nematologists.

AB Two β-1,4-endoglucanase (EGase) cDNAs were isolated from Globodera tabacum, the tobacco cyst nematode, and have been designated as GT-eng-1 and GT-eng-2. GT-eng-1 and GT-eng-2 encode precursor proteins with a predicted secretion signal sequence, cellulolytic **catalytic domain**, and a **linker** domain. The protein product GT-ENG-1 contains an addnl. 95 amino acid carboxy terminal sequence with strong similarity to type II **cellulose binding domains**. Riboprobes and polyclonal antibodies raised to recombinant cyst nematode EGases were used to follow expression patterns of EGase transcripts and proteins throughout the nematode life cycle. EGase transcripts and proteins were specifically detected within the subventral esophageal gland cells of G.

tabacum second-stage juveniles (J2) within eggs prior to hatching, in preparasitic J2, and in parasitic J2 that had invaded tobacco roots. EGase transcripts and proteins were not detected in G. tabacum after the molt to the sedentary J3, J4, and adult female life stages. Interestingly, EGase transcription and translation resumed in the subventral esophageal glands of late J4 males. It is hypothesized that secreted EGases play a major role to facilitate intracellular migration of G. tabacum within tobacco roots.

L21 ANSWER 17 OF 60 CAPLUS COPYRIGHT 2004 ACS on STN
2000:623708 Document No. 133:219465 Genomic, cDNA and deduced amino acid sequences of cellulase CelF of the anaerobic rumen fungus Orpinomyces PC-2. Li, Xin-liang; Chen, Huizhong; Ljungdahl, Lars G. (University of Georgia Research Foundation, Inc., USA). U.S. US 6114158 A 20000905, 24 pp. (English). CODEN: USXXAM. APPLICATION: US 1998-118319 19980717.
AB A cDNA (1,520 bp), designated celF, consisting of an open reading frame (ORF) encoding a novel cellulase CelF (cellobiohydrolase) of 432 amino acids was isolated from a cDNA library of the anaerobic rumen fungus Orpinomyces PC-2 constructed in Escherichia coli. Anal. of the deduced amino acid sequence showed that starting from the N-terminus, CelF consists of a signal peptide, a **cellulose binding domain** (CBD) followed by an extremely Asn-rich **linker** region which sep. the CBD and the **catalytic domains**. The latter is located at the C-terminus. The **catalytic domain** of CelF is highly homologous to CelA and CelC of Orpinomyces PC-2, to CelA of Neocallimastix patriciarum and also to cellobiohydrolase IIs (CBHIIs) from aerobic fungi. However, Like CelA of Neocallimastix patriciarum, CelF does not have the noncatalytic repeated peptide domain (NCRPD) found in CelA and CelC from the same organism. The recombinant protein CelF hydrolyzes cellooligosaccharides in the pattern of CBHII, yielding only cellobiose as product with cellotetraose as the substrate. The gene celF is interrupted by a 111 bp intron, located within the region coding for the CBD. The intron of the celF has features in common with genes from aerobic filamentous fungi.

L21 ANSWER 18 OF 60 CAPLUS COPYRIGHT 2004 ACS on STN
2000:327223 Structure/function studies for the construction of a cellulolytic enzyme mimetic.. Mosier, Nathan S.; Sarikaya, Ayda; Ladisch, Michael R. (Laboratory of Renewable Resources Engineering and Department of Agricultural and Biological Engineering, Purdue University, West Lafayette, IN, 47907, USA). Book of Abstracts, 219th ACS National Meeting, San Francisco, CA, March 26-30, 2000, BIOT-004. American Chemical Society: Washington, D. C. (English) 2000. CODEN: 69CLAC.
AB Technol. and economic hurdles preventing the widespread use of current methods for cellulose hydrolysis by dilute acid hydrolysis or enzymic hydrolysis have prompted the search for alternate methods of saccharifying biomass. This research focuses on using the new information on the structural and functional properties of cellulolytic enzymes available in the literature to develop an organic mol. that will mimic the functional characteristics of cellulolytic enzymes while overcoming the economic barrier of current enzyme technol. Cellulolytic enzymes contain three distinct structural/functional components, a **catalytic domain**, a **cellulose binding domain**, and a **linker** region joining the other two regions. This paper addresses the initial steps for the construction of a cellulolytic enzyme mimetic. Candidates for a catalytic moiety have been screened for β 1-4 glycosidic bond hydrolysis as well as glucose degradation Candidates for a **cellulose binding domain** have also been screened using a rapid chromatog. method.

L21 ANSWER 19 OF 60 CAPLUS COPYRIGHT 2004 ACS on STN
1999:765831 Document No. 132:118144 Characterization of a Neocallimastix

patriciarum xylanase gene and its product. Liu, Jin-Hao; Selinger, Brent L.; Tsai, Cheng-Fang; Cheng, Kuo-Jaon (Institute of BioAgricultural Sciences, Academia Sinica, Taipei, 11529, Taiwan). Canadian Journal of Microbiology, 45(11), 970-974 (English) 1999. CODEN: CJMIAZ. ISSN: 0008-4166. Publisher: National Research Council of Canada.

AB A xylanase gene (xynC) isolated from the anaerobic ruminal fungus Neocallimastix patriciarum was characterized. The gene consists of an N-terminal **catalytic domain** that exhibited homol. to family 11 of glycosyl hydrolases, a C-terminal **cellulose binding domain** (CBD) and a putative dockerin domain in between. Each domain was linked by a short **linker** domain rich in proline and alanine. Deletion anal. demonstrated that the CBD was essential for optimal xylanase activity of the enzyme, while the putative dockerin domain may not be required for enzyme function.

L21 ANSWER 21 OF 60 CAPLUS COPYRIGHT 2004 ACS on STN

1999:614319 Document No. 131:297084 The type II and X **cellulose-binding domains** of Pseudomonas xylanase A potentiate catalytic activity against complex substrates by a common mechanism. Gill, Jaitinder; Rixon, Jane E.; Bolam, David N.; McQueen-Mason, Simon; Simpson, Peter J.; Williamson, Michael P.; Hazlewood, Geoffrey P.; Gilber, Harry J. (Department of Biological and Nutritional Sciences, University of Newcastle upon Tyne, Newcastle upon Tyne, NE1 7RU, UK). Biochemical Journal, 342(2), 473-480 (English) 1999. CODEN: BIJOAK. ISSN: 0264-6021. Publisher: Portland Press Ltd..

AB Xylanase A (Pf Xyn10A), in common with several other Pseudomonas fluorescens subsp. cellulosa polysaccharidases, consists of a Type II **cellulose-binding domain** (CBD), a **catalytic domain** (Pf Xyn10ACD) and an internal domain that exhibits homol. to Type X CBDs. The Type X CBD of Pf Xyn10A, expressed as a discrete entity (CBDX) or fused to the **catalytic domain** (Pf Xyn10A'), bound to amorphous and bacterial microcryst. cellulose with a Ka of 2.5 + 105 M-1. CBDX exhibited no affinity for soluble forms of cellulose or cello-oligosaccharides, suggesting that the domain interacts with multiple cellulose chains in the insol. forms of the polysaccharide. Pf Xyn10A' was 2-3 times more active against cellulose-hemicellulose complexes than Pf Xyn10ACD; however, Pf Xyn10A' and Pf Xyn10ACD exhibited the same activity against soluble substrates. CBDX did not disrupt the structure of plant-cell-wall material or bacterial microcryst. cellulose, and did not potentiate Pf Xyn10ACD when not covalently linked to the enzyme. There was no substantial difference in the affinity of full-length Pf Xyn10A and the enzyme's Type II CBD for cellulose. The activity of Pf Xyn10A against cellulose-hemicellulose complexes was similar to that of Pf Xyn10A', and a derivative of Pf Xyn10A in which the Type II CBD is linked to the Pf Xyn10ACD via a serine-rich **linker** sequence [Bolam, Cireula, McQueen-Mason, Simpson, Williamson, Rixon, Boraston, Hazlewood and Gilbert (1998) Biochem. J. 331, 775-781]. These data indicate that CBDX is functional in Pf Xyn10A and that no synergy, either in ligand binding or in the potentiation of catalysis, is evident between the Type II and X CBDs of the xylanase.

L21 ANSWER 23 OF 60 CAPLUS COPYRIGHT 2004 ACS on STN

1999:494167 Document No. 131:268905 Functional analysis of the carbohydrate-binding domains of Erwinia chrysanthemi Cel5 (endoglucanase Z) and an Escherichia coli putative chitinase. Simpson, Helen D.; Barras, Frederic (Laboratoire de Chimie Bacterienne, Centre National de la Recherche Scientifique, Marseille, 13402, Fr.). Journal of Bacteriology, 181(15), 4611-4616 (English) 1999. CODEN: JOBAAY. ISSN: 0021-9193. Publisher: American Society for Microbiology.

AB The Cel5 cellulase (formerly known as endoglucanase Z) from Erwinia chrysanthemi is a multidomain enzyme consisting of a **catalytic domain**, a

linker region, and a **cellulose binding domain** (CBD). A three-dimensional structure of the CBDCel5 has previously been obtained by NMR. In order to define the role of individual residues in cellulose binding, site-directed mutagenesis was performed. The role of three aromatic residues (Trp18, Trp43, and Tyr44) in cellulose binding was demonstrated. The exposed potential hydrogen bond donors, residues Gln22 and Glu27, appeared not to play a role in cellulose binding, whereas residue Asp17 was found to be important for the stability of Cel5. A deletion mutant lacking the residues Asp17 to Pro23 bound only weakly to cellulose. The sequence of CBDCel5 exhibits homol. to a series of five repeating domains of a putative large protein, referred to as Yheb, from Escherichia coli. One of the repeating domains (Yheb1), consisting of 67 amino acids, was cloned from the E. coli chromosome and purified by metal chelating chromatog. While CBDCel5 bound to both cellulose and chitin, Yheb1 bound well to chitin, but only very poorly to cellulose. The Yheb protein contains a region that exhibits sequence homol. with the **catalytic domain** of a chitinase, which is consistent with the hypothesis that the Yheb protein is a chitinase.

L21 ANSWER 27 OF 60 CAPLUS COPYRIGHT 2004 ACS on STN

1999:91347 Cellulase core proteins from trichoderma reesei; binding properties and efficiency in cellulose hydrolysis. Tenkanen, Maija; Suurnakki, Anna; Siika-aho, Matti; Palonen, Hetti; Linder, Markus; Kotiranta, Pia; Tjerneld, Folke; Buchert, Johanna; Viikari, Liisa (VTT Biotechnology and Food Research, FIN-02044, Finland). Book of Abstracts, 217th ACS National Meeting, Anaheim, Calif., March 21-25, CELL-018. American Chemical Society: Washington, D. C. (English) 1999. CODEN: 67GHA6.

AB The main four cellulases of Trichoderma reesei, cellobiohydrolase I and II (CBH I, II) and endoglucanases I and II (EG I, II), have a modular structure consisting of a **catalytic domain** and a **cellulose binding domain** (CBD) which are separated by a **linker** peptide. **Cellulose binding domain** is know to enhance the activity of cellulases, especially cellobiohydrolases, on crystalline cellulose. The binding of CBDCBHI on cellulose has recently been shown to be reversible. The binding of whole cellulases and their core proteins on cellulose was compared in the present work. The enzymes were labeled with tritium which enabled the accurate quantification of low concns. of proteins. All enzymes retained their activity after labeling. The action of whole cellulases and corresponding core proteins were also compared in the hydrolysis of pure cellulose and kraft pulp. In addition the effect of these proteins on pulp viscosity and paper tech. properties was evaluated.

L21 ANSWER 29 OF 60 CAPLUS COPYRIGHT 2004 ACS on STN

1998:382642 Document No. 129:133020 Multidomain structure and cellulosomal localization of the Clostridium thermocellum cellobiohydrolase CbhA. Zverlov, Vladimir V.; Velikodvorskaya, Galina V.; Schwarz, Wolfgang H.; Bronnenmeier, Karin; Kellermann, Josef; Staudenbauer, Walter L. (Institute of Molecular Genetics, Russian Academy of Science, Moscow, 123182, Russia). Journal of Bacteriology, 180(12), 3091-3099 (English) 1998. CODEN: JOBAAY. ISSN: 0021-9193. Publisher: American Society for Microbiology.

AB The nucleotide sequence of the Clostridium thermocellum F7 cbhA gene, coding for the cellobiohydrolase CbhA, has been determined An open reading frame encoding a protein of 1230 amino acids was identified. Removal of a putative signal peptide yields a mature protein of 1203 amino acids with a mol. weight of 135,139. Sequence anal. of CbhA reveals a multidomain structure of unusual complexity consisting of an N-terminal **cellulose binding domain** (CBD) homologous to CBD family IV, an Ig-like β-barrel domain, a **catalytic domain** homologous to cellulase family E1, a duplicated domain similar to fibronectin type III (Fn3) modules, a CBD homologous to family III, a highly acidic **linker**

region, and a C-terminal dockerin domain. The cellulosomal localization of CbhA was confirmed by Western blot anal. employing polyclonal antibodies raised against a truncated enzymically active version of CbhA. CbhA was identified as cellulosomal subunit S3 by partial amino acid sequence anal. Comparison of the multidomain structures indicates striking similarities between CbhA and a group of cellulases from actinomycetes. Average linkage cluster anal. suggests a coevolution of the N-terminal CBD and the **catalytic domain** and its spread by horizontal gene transfer among gram-pos. cellulolytic bacteria.

L21 ANSWER 31 OF 60 CAPLUS COPYRIGHT 2004 ACS on STN

1998:163676 Document No. 128:214198 Cloning and gene sequence of novel endoglucanases from Cellvibrio mixtus and C. gilvus. Bjornvad, Mads Eskelund; Nielsen, Preben (Novo Nordisk A/S, Den.; Bjornvad, Mads Eskelund; Nielsen, Preben). PCT Int. Appl. WO 9808940 A1 19980305, 118 pp. DESIGNATED STATES: W: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG. (English). CODEN: PIXXD2. APPLICATION: WO 1997-DK348 19970826. PRIORITY: DK 1996-893 19960826; DK 1996-1015 19960917.

AB An enzyme preparation consisting essentially of an endo-β-1,4-glucanase (EC 3.2.1.4) derived from the bacterial genera Cellvibrio mixtus or Cellvibrio gilvus is produced by recombinant techniques using a cloned DNA sequence encoding the enzyme. Endo-β-1,4-glucanase from C. mixtus DM 1523 comprises a gene-deduced sequence of 527 amino acids, including a signal peptide of 32 amino acid residues, a **cellulose- binding domain** belonging to family IIa (residues 33-134), a serine-rich **linker** (135-185), a **cellulose- binding domain** belonging to family X (186-234), a second serine-rich **linker** (235-277), and a **catalytic domain** (residues 278 to the end) belonging to family 45 of the glycosyltransferases. The endo-β-1,4-glucanase has 2 conserved regions, a first amino acid sequence consisting of 15 amino acid residues having sequence and a second amino acid sequence consisting of 6 amino acid residues having sequence, and is useful in industrial application conventionally using cellulolytic enzymes. Techniques are described for constructing a hybrid endoglucanase comprising the C. mixtus cel45 core with Humicola insolens EG V **linker** and CBD, and for transformation and expression of the Cellvibrio enzyme in Pseudomonas fluorescens and P. cepacia.

L21 ANSWER 33 OF 60 CAPLUS COPYRIGHT 2004 ACS on STN

1998:76000 Document No. 128:151117 Improved thermostability in cellulase by production of the C-terminal truncated **catalytic domain** . Adney, William S.; Thomas, Steven R.; Baker, John O.; Himmel, Michael E.; Chou, Yat-Chen (Midwest Research Institute, USA). U.S. US 5712142 A 19980127, 19 pp., Cont.-in-part of U.S. 5,536,655. (English). CODEN: USXXAM. APPLICATION: US 1996-604913 19960222. PRIORITY: US 1989-412434 19890926; US 1992-826089 19920127; US 1993-125115 19930921; US 1994-276213 19940715.

AB The gene encoding Acidothermus cellulolyticus E1 endoglucanase is cloned and expressed in Pichia pastoris. A new modified E1 endoglucanase enzyme comprising the **catalytic domain** (residues 1-358) of the full-size, mature E1 enzyme demonstrates enhanced thermostability and is produced by 2 methods. The first method of producing the new modified E1 is proteolytic cleavage to remove the **cellulose binding domain** and **linker** peptide of the full size E1.

The second method of producing the new modified E1 is genetic truncation of the gene encoding the full size E1 so that the **catalytic domain** is expressed in the expression product.

L21 ANSWER 35 OF 60 CAPLUS COPYRIGHT 2004 ACS on STN

1997:789845 Document No. 128:112204 Two cellulases, CelA and CelC, from the polycentric anaerobic fungus Orpinomyces strain PC-2 contain N-terminal docking domains for a cellulase-hemicellulase complex. Li, Xin-Liang; Chen, Huizhong; Ljungdahl, Lars G. (Department of Biochemistry and Molecular Biology and Center for Biological Resource Recovery, The University of Georgia, Athens, GA, 30602-7229, USA). Applied and Environmental Microbiology, 63(12), 4721-4728 (English) 1997. CODEN: AEMIDF. ISSN: 0099-2240. Publisher: American Society for Microbiology.

AB Two cDNAs encoding two cellulases, CelA and CelC, were isolated from a cDNA library of the polycentric anaerobic fungus Orpinomyces sp. strain PC-2 constructed in Escherichia coli. Nucleotide sequencing revealed that the celA cDNA (1,558 bp) and celC cDNA (1,628 bp) had open reading frames encoding polypeptides of 459 (CelA) and 449 (CelC) amino acids, resp. The two cDNAs were 76.9 and 67.7% identical at the nucleotide and amino acid levels, resp. Anal. of the deduced amino acid sequences showed that starting from the N termini, both CelA and CelC had signal peptides, which were followed by noncatalytic repeated peptide domains (NCRPD) containing two repeated sequences of 33 to 40 amino acid residues functioning as docking domains. The NCRPDs and the **catalytic domains** were separated by **linker** sequences. The NCRPDs were homologous to those found in several hydrolases of anaerobic fungi, whereas the **catalytic domains** were homologous to the **catalytic domains** of fungal cellobiohydrolases and bacterial endoglucanases. The **linker** sequence of CelA contained predominantly glutamine and proline residues, while that of CelC contained mainly threonine residues. CelA and CelC did not have a typical **cellulose binding domain** (CBD). CelA and CelC expressed in E. coli rapidly decreased the viscosity of CM-cellulose (CMC), indicating that there was endoglucanase activity. In addition, they produced cellobiose from CMC, acid-swollen cellulose, and cellotetraose, suggesting that they had cellobiohydrolase activity. The optimal activity conditions with CMC as the substrate were pH 4.3 to 6.8 and 50°C for CelA and pH 4.6 to 7.0 and 40°C for CelC. Despite the lack of a CBD, CelC displayed a high affinity for microcryst. cellulose, whereas CelA did not.

L21 ANSWER 36 OF 60 CAPLUS COPYRIGHT 2004 ACS on STN

1997:738065 Document No. 128:72479 Purification of the Ruminococcus albus endoglucanase IV using a **cellulose-binding domain** as an affinity tag. Karita, Shuichi; Kimura, Tetsuya; Sakka, Kazuo; Ohmiya, Kunio (Center for Molecular Biology and Genetics, Mie University, Tsu, 514, Japan). Journal of Fermentation and Bioengineering, 84(4), 354-357 (English) 1997. CODEN: JFBIEX. ISSN: 0922-338X. Publisher: Society for Fermentation and Bioengineering, Japan.

AB The gene encoding the single **cellulose-binding domain** II (CBD II) of Clostridium stercorarium xylanase A was fused to the egIV gene encoding endoglucanase IV (EGIV) from Ruminococcus albus. The fusion protein (EGIV + CBDII) expressed in Escherichia coli could be readily purified from the cell-free extract of E. coli in a single step using the affinity of CBD to cellulose. The purified enzyme was cleaved into two moieties, i.e. the **catalytic domain** and CBD, at a specific site in the **linker** region by partial digestion with trypsin at 4°C. This result indicates that this CBD belonging to family VI of the CBD families can be used as an affinity tag for purification of the recombinant protein.

L21 ANSWER 37 OF 60 CAPLUS COPYRIGHT 2004 ACS on STN
1997:589589 Document No. 127:274469 Cloning, expression in Streptomyces lividans and biochemical characterization of a thermostable endo-β-1,4-xylanase of Thermomonospora alba ULJB1 with cellulose-binding ability. Blanco, J.; Coque, J. J. R.; Velasco, J.; Martin, J. F. (Faculty of Biology, Area of Microbiology, University of Leon, Leon, 24071, Spain). Applied Microbiology and Biotechnology, 48(2), 208-217 (English) 1997. CODEN: AMBIDG. ISSN: 0175-7598. Publisher: Springer.

AB Several thermophilic actinomycetes were isolated from urban solid waste. One of them, Thermomonospora alba ULJB1, showed a broad degradative activity on xylan, cellulose, starch and other polymers. Xylanase and cellulase activities were quantified and compared with those of Thermomonospora fusca. Genes encoding two different endo-β-1,4- xylanases were cloned from T. alba ULJB1. One of them, xylA, was sequenced, subcloned and overexpressed in Streptomyces lividans. It encodes a protein of 482 amino acids with a deduced mol. mass of 48 456 Da. The protein contains a 38-amino-acid leader peptide with six Arg+ residues in its amino-terminal end, a **catalytic domain** and a **cellulose-binding domain** connected by a **linker** region rich in proline and glycine. The XylA protein was purified to near homogeneity from S. lividans/xylA cultures. Two forms of the extracellular xylanase, of 48 kDa and 38 kDa, were produced that differed in their cellulose-binding ability. The 48-kDa protein showed a strong binding to cellulose whereas the 38-kDa form did not bind to this polymer, apparently because of the removal during processing of the **cellulose-binding domain**. Both forms were able to degrade xylans form different origins but not lichenan or CM-cellulose. The major degradation product was xylobiose with traces of xylose. The xylanase activity was thermostable, showing a good activity up to 95°, and had broad pH stability in the range from pH 4.0 to pH 10.0.

L21 ANSWER 39 OF 60 CAPLUS COPYRIGHT 2004 ACS on STN
1997:120969 Document No. 126:187540 Do the non-catalytic polysaccharide-binding domains and **linker** regions enhance the biobleaching properties of modular xylanases?. Rixon, J. E.; Clarke, J. H.; Hazlewood, G. P.; Hoyland, R. W.; McCarthy, A. J.; Gilbert, H. J. (Dep. Biological Nutritional Sciences, University Newcastle upon Tyne, Newcastle upon Tyne, NE1 7RU, UK). Applied Microbiology and Biotechnology, 46(5/6), 514-520 (English) 1996. CODEN: AMBIDG. ISSN: 0175-7598. Publisher: Springer.

AB Xylanase A (XylA) from Pseudomonas fluorescens subsp. cellulosa consists of an N-terminal non-catalytic **cellulose-binding domain** joined to a functionally independent C-terminal **catalytic domain** by a sequence rich in serine residues. Xylanase D (XylD) from Cellulomonas fimi also exhibits a modular structure comprising an N-terminal **catalytic domain** linked to an internal non-catalytic xylan-binding domain and a C-terminal **cellulose-binding domain**. To determine the importance of the non-catalytic polysaccharide-binding domains and **linker** sequences of XylA and XylD in relation to their capacity to hydrolyze pulp xylan and enhance bleachability, purified full-length and modified derivs. of both enzymes were incubated with a hardwood kraft pulp. Deletion of the **cellulose-binding domain** or **linker** region from XylA decreased the activity of the enzyme against pulp xylan, but had no significant effect on the capacity of the enzyme to facilitate delignification and reduce pulp kappa number While full-length and truncated forms of XylD, lacking either the cellulose-binding or the cellulose- and xylan-binding domains, were equally effective in hydrolyzing pulp xylan, enzyme derivs. containing a polysaccharide-binding domain were marginally more efficient in reducing pulp kappa number The reduction in kappa number elicited by full-length and isolated **catalytic domains** of XylA and XylD was reflected in an increase in the brightness of paper handsheets derived from pretreated pulps. Thus, the polysaccharide-

binding domains of XylA and XylD did not appear to confer any advantage in terms of the ability of the enzymes to improve pulp bleachability. However, XylA and XylD, which belong to different glycosyl hydrolase families, differed in their ability to hydrolyze pulp xylan and facilitate the delignification of kraft pulp.

L21 ANSWER 41 OF 60 CAPLUS COPYRIGHT 2004 ACS on STN
1996:646954 Document No. 125:296089 Evidence that **linker** sequences and **cellulose-binding domains** enhance the activity of hemicellulases against complex substrates. Black, Gary W.; Rixon, Jane E.; Clarke, Jonathan H.; Hazlewood, Geoffrey P.; Theodorou, Michael K.; Morris, Philip; Gilbert, Harry J. (Dep. Biological, Univ. Newcastle upon Tyne, Newcastle upon Tyne, NE1 7RU, UK). Biochemical Journal, 319(2), 515-520 (English) 1996. CODEN: BIJOAK. ISSN: 0264-6021. Publisher: Portland Press.

AB Xylanase A (XYLA) and arabinofuranosidase C (XYLC) from Pseudomonas fluorescens subsp. cellulosa are modular enzymes consisting of discrete **cellulose-binding domains** (CBDs) and **catalytic domains** joined by serine-rich **linker** sequences. To evaluate the role of the CBDs and interdomain regions, the capacity of full-length and truncated derivs. of the two enzymes, lacking either the **linker** sequences or CBDs, to hydrolyze a range of substrates, and bind to cellulose, was determined Removal of the CBDs did not affect either the activity of XYLA or XYLC against soluble arabinoxylan. Similarly, deletion of the **linker** sequences did not alter the affinity of the enzymes for cellulose or their activity against soluble substrates, even when bound to cellulose via the CBDs. Truncated derivs. of XYLA lacking either the **linker** sequences or the CBD were less active against xylan contained in cellulose-hemicellulose complexes, compared with the full-length xylanase. Similarly, removal of the CD from XYLC diminished the activity of the enzyme (XYLC''') against plant-cell-wall material containing highly substituted arabinoxylan. The role of CBDs and **linker** sequences in the catalytic activity of hemicellulases against the plant cell wall is discussed.

L21 ANSWER 44 OF 60 CAPLUS COPYRIGHT 2004 ACS on STN
1995:728078 Document No. 123:279385 The non-catalytic **cellulose-binding domain** of a novel cellulase from Pseudomonas fluorescens subsp. cellulosa is important for the efficient hydrolysis of Avicel. Hall, Judith; Black, Gary W.; Ferreira, Luis M. A.; Millward-Sadler, Sarah J.; Ali, Bassam R. S.; Hazlewood, Geoffrey P.; Gilbert, Harry J. (Dep. Biol. Nutr. Sci., Univ. Newcastle upon Tyne, Newcastle upon Tyne, NE1 7RU, UK). Biochemical Journal, 309(3), 749-56 (English) 1995. CODEN: BIJOAK. ISSN: 0264-6021. Publisher: Portland Press.

AB A genomic library of Pseudomonas fluorescens subsp. cellulosa DNA, constructed in λZAPII, was screened for carboxymethyl-cellulase activity. The pseudomonad insert from a recombinant phage which displayed elevated cellulase activity in comparison with other cellulase-pos. clones present in the library, was excised into pBluescript SK- to generate the plasmid pC48. The nucleotide sequence of the cellulase gene, designated celE, revealed a single open reading frame of 1710 bp that encoded a polypeptide, defined as endoglucanase E (CelE), of Mr 59,663. The deduced primary structure of CelE revealed an N-terminal signal peptide followed by a 300-amino-acid sequence that exhibited significant identity with the **catalytic domains** of cellulases belonging to glycosyl hydrolase Family 5. Adjacent to the **catalytic domain** was a 40-residue region that exhibited strong sequence identity to non- **catalytic domains** located in 2 other endoglucanases and a xylanase from P. fluorescens. The C-terminal 100 residues of CelE were similar to Type-I **cellulose-binding domains** (CBDs). The 3 domains of the cellulase were joined by **linker**

sequences rich in serine residues. Anal. of the biochem. properties of full-length and truncated derivs. of CelE confirmed that the enzyme comprised an N-terminal **catalytic domain** and a C-terminal CBD. Anal. of purified CelE revealed that the enzyme had an Mr of 56,000 and an exptl. determined N-terminal sequence identical to residues 40-54 of the deduced primary structure of full-length CelE. The enzyme exhibited an endo mode of action in hydrolyzing a range of cellulosic substrates including Avicel and acid-swollen cellulose, but did not attack xylan or any other hemicelluloses. A truncated form of the enzyme, which lacked the C-terminal CBD, displayed the same activity as full-length CelE against soluble cellulose and acid-swollen cellulose, but exhibited substantially lower activity than the full-length cellulase against Avicel. The significance of these data in relation to the role of the CBD is discussed.

L21 ANSWER 45 OF 60 CAPLUS COPYRIGHT 2004 ACS on STN

1995:639916 Document No. 123:189785 celA, another gene coding for a multidomain cellulase from the extreme thermophile Caldocellum saccharolyticum. Te'o, V. S. J.; Saul, D. J.; Bergquist, P. L. (Sch. Biol. Sci., Univ. Auckland, Auckland, 92019, N. Z.). Applied Microbiology and Biotechnology, 43(2), 291-6 (English) 1995. CODEN: AMBIDG. ISSN: 0175-7598. Publisher: Springer.

AB Caldocellum saccharolyticum is an extremely thermophilic anaerobic bacterium capable of growth on cellulose and hemicellulose as sole carbon sources. Cellulase and hemicellulase genes have been found clustered together on its genome. The gene for one of the cellulases (celA) was isolated on a λ genomic library clone, sequenced and found to comprise a large open-reading frame of 5253 base pairs that could be translated into a peptide of 1751 amino acids. To date, it is the largest cellulase gene sequenced. The translated product is a multidomain structure composed of two **catalytic domains** and two **cellulose-binding domains** linked by proline-threonine-rich regions (PT **linkers**). The N-terminal domain of celA encodes for an endoglucanase activity on CM-cellulose, consistent with its high homol. to the sequences of several other endo-1,4-β-D-glucanases. The carboxyterminal domain shows sequence homol. with a cellulase from Clostridium thermocellum (CelS), which is known to act synergistically with a second component to hydrolyze crystalline cellulose. In the absence of a Caldocellum homolog for this second protein, the authors detected no activity from this domain.

L21 ANSWER 46 OF 60 CAPLUS COPYRIGHT 2004 ACS on STN

1995:280498 Document No. 122:206597 Cloning and sequencing of an endo-β-1,4-glucanase gene mcenA from Micromonospora cellulolyticum 86W-16. Lin, Feng; Marchenko, George; Cheng, Yuan-Rong (Fujian Institute Microbiology, Fuzhou, 350007, Peop. Rep. China). Journal of Industrial Microbiology, 13(6), 344-50 (English) 1994. CODEN: JIMIE7. ISSN: 0169-4146. Publisher: Stockton.

AB Endo-β-1,4-glucanase gene mcenA of Micromonospora cellulolyticum 85W-16 was cloned, and the nucleotide sequence was determined An open reading frame (ORF) of 1374 bases, coding for a peptide (McenA) of 457 amino acids and 46,742 Da, was found. It is preceded by a Gram-pos. type of ribosome-binding site and followed by an imperfect inverted repeat. A putative signal peptide containing 23 amino acids is at the N-terminus and a **linker** region possessing 37 amino acids is in the midpart of McenA. The N-half of McenA functions as the **catalytic domain** and the C-half might serve as a **cellulose- binding domain** (CBD). Deletion of the latter did not decrease the CMCase activity of McenA. Significant similarity (705) was found between the amino acid sequences of McenA and MbcelA, an endoglucanase from Microbispora bispora.

L21 ANSWER 47 OF 60 CAPLUS COPYRIGHT 2004 ACS on STN
1995:225668 Document No. 122:181429 Structure-function studies of endo-1,4-β-D-glucanase E2 from Thermomonospora fusca. Spezio, Mike; Karplus, P. Andrew; Irwin, Diana; Wilson, David B. (Section Biochem., Cornell Univ., Ithaca, NY, 14853, USA). ACS Symposium Series, 566(Enzymatic Conversion of Biomass for Fuels Production), 66-74 (English) 1994. CODEN: ACSMC8. ISSN: 0097-6156. Publisher: American Chemical Society.

AB A review with 21 refs. Six different cellulases have been purified to homogeneity from the culture supernatant of a protease neg. mutant of the soil bacterium, Thermomonospora fusca. Three of these enzymes are endoglucanases, two are exocellulases, and one is an exocellulase with some endoglucanase activity. All six enzymes contain a **cellulose -binding domain** joined by a flexible **linker** to a **catalytic domain**. When the distributions of reducing sugars between the solution and filter paper in filter paper assays of E2 and E2cd were determined, there were two soluble sugars produced for each insol. sugar with E2, whereas with E2cd there was only one soluble sugar produced for each insol. sugar. This result seems reasonable since the **cellulose-binding domain** should attach the enzyme to a specific site on the substrate for a longer time than would the **catalytic domain** alone.

L21 ANSWER 48 OF 60 CAPLUS COPYRIGHT 2004 ACS on STN
1995:219665 Document No. 122:75918 Purification and processing of **cellulose-binding domain**-alkaline phosphatase fusion proteins. Greenwood, Jeffrey M.; Gilkes, Neil R.; Miller, Robert C., Jr.; Kilburn, Douglas G.; Warren, R. Antony J. (Department of Microbiology and Immunology and Protein Engineering, University of British Columbia, Vancouver, BC, V6T 1Z3, Can.). Biotechnology and Bioengineering, 44(11), 1295-305 (English) 1994. CODEN: BIBIAU. ISSN: 0006-3592. Publisher: Wiley.

AB Fusion of the leader peptide and the **cellulose-binding domain** (CBD) of endoglucanase A (CenA) from Cellulomonas fimi, with or without **linker** sequences, to the N-terminus of alkaline phosphatase (PhoA) from Escherichia coli leads to the accumulation of significant amts. of the CBD-PhoA fusion proteins in the supernatants of E. coli cultures. The fusion proteins can be purified from the supernatants by affinity chromatog. on cellulose. The fusion proteins can be desorbed from the cellulose with water or guanidine-HCl. If the sequence IEGR is present between the CBD and PhoA, the CBD can be cleaved from the Phoa with factor Xa. The efficiency of hydrolysis by factor Xa is strongly influenced by the amino acids on either side of the IEGR sequence. The CBD released by factor Xa is removed by adsorption to cellulose. A nonspecific protease from C. fimi, which hydrolyzes native CenA between the CBD and the **catalytic domain**, may be useful for removing the CBD from some fusion proteins.

L21 ANSWER 50 OF 60 CAPLUS COPYRIGHT 2004 ACS on STN
1994:476764 Document No. 121:76764 Structure and functions of cellulases. Ohmiya, Kunio; Sakka, Kazuo (Fac. Bioresour., Mie Univ., Tsu, 514, Japan). Kagaku to Seibutsu, 32(6), 373-80 (Japanese) 1994. CODEN: KASEAA. ISSN: 0453-073X.

AB A review, with 32 refs., on cellulase complex cellulosome, **catalytic domains** and **cellulose-binding domains** of cellulase, amino acid sequences of **linkers** of bacterial cellulase, crystal anal. of cellulase, and future aspects on study of cellulase.

L21 ANSWER 54 OF 60 CAPLUS COPYRIGHT 2004 ACS on STN
1994:48515 Document No. 120:48515 Genetic analysis of cellulases. Ohmiya, Kunio; Karita, Shuichi; Sakka, Kazuo; Shimada, Kyo (Fac. Bioresources, Mie Univ., Kamihamacho, 513, Japan). Microbial Utilization of Renewable Resources, Volume Date 1992, 8, 162-81 (English) 1993. CODEN: MURRE6.
AB A review, with 147 refs., on cellulases and xylanases. Topics discussed include **catalytic domains**, **cellulose- binding domains**, **linkers** of the domains, and enzyme properties.

L21 ANSWER 55 OF 60 CAPLUS COPYRIGHT 2004 ACS on STN
1993:423579 Document No. 119:23579 Sequence analysis of a gene cluster encoding cellulase from Clostridium cellulolyticum. Bagnara-Tardif, Chantal; Gaudin, Christian; Belaich, Anne; Hoest, Philippe; Citard, Thierry; Belaich, Jean-Pierre (Lab. Chim. Bact., CNRS, Marseille, Fr.). Gene, 119(1), 17-28 (English) 1992. CODEN: GENED6. ISSN: 0378-1119.
AB The sequence of a 5633-bp EcoRI-PvuII DNA fragment from C. cellulolyticum was determined This fragment contains 2 complete endo-β-1,4-glucanase- encoding genes, designated celCCC and celCCG. These 2 genes are flanked by 2 other partial open reading frames (ORF1 and celCCE) that probably encode 2 cellulases or related enzymes. The celCCC and celCCG genes appear to be present in a polycistronic transcriptional unit. Northern blot hybridizations with intragenic probes derived from celCCC and celCCG gave similar patterns. Two transcripts of about 5 and 6 kb were identified. The celCCC and celCCG ORFs extend over 1380 bp and 2175 bp, resp. They are separated by only 87 nucleotides. A typical signal sequence is present at the N terminus of the deduced polypeptides. The mature CelCCC and CelCCG proteins have Mrs 47,201 and 76,101, resp. Their amino acid (aa) sequences were compared to other known cellulase sequences. Both contain the repeated 24-aa sequence characteristic of clostridial β-glycanases. The N-terminal **catalytic domains** of CelCCC and CelCCG can be classified into the D and E2 families, resp. Finally, CelCCG contains an addnl. internal domain which is very similar to that of the Bacillus-type **cellulose-binding domain** (CBD). The ORF1 C-terminal-encoded sequence also contains the clostridial 24-aa repeat. The CelCCE N-terminus consists of a typical signal sequence followed by a 168-aa domain homologous to the N-terminal repeated domain of Cellulomonas fimi CenC. This domain is connected to an incomplete **catalytic domain** of family E1 by a Pro-rich junction **linker**.

L21 ANSWER 56 OF 60 CAPLUS COPYRIGHT 2004 ACS on STN
1993:250611 Document No. 118:250611 Cellulose-binding polypeptides from Cellulomonas fimi: endoglucanase D (CenD), a family A β-1,4-glucanase. Meinke, A.; Gilkes, N. R.; Kilburn, D. G.; Miller, R. C, Jr.; Warren, R. A. J. (Dep. Microbiol., Univ. British Columbia, Vancouver, BC, V6T 1Z3, Can.). Journal of Bacteriology, 175(7), 1910-18 (English) 1993. CODEN: JOBAAY. ISSN: 0021-9193.
AB Five cellulose-binding polypeptides were detected in C. fimi culture supernatants. Two of them are CenA and CenB, endo-β-1,4-glucanases which have been characterized previously; the other three were previously uncharacterized polypeptides with apparent mol. masses of 120, 95, and 75 kDa. The 75-kDa cellulose-binding protein was designated endoglucanase D (CenD). The cenD gene was cloned and sequenced. It encodes a polypeptide of 747 amino acids. Mature CenD is 708 amino acids long and has a predicted mol. mass of 74,982 Da. Anal. of the predicted amino acid sequence of CenD shows that the enzyme comprises four domains which are separated by short **linker** polypeptides: an N-terminal **catalytic domain** of 405 amino acids, two repeated sequences of 95 amino acids each, and a C-terminal domain of 105 amino acids which is >50% identical to the sequences of **cellulose- binding domains** in Cex, CenA, and

CenB from C. fimi. Amino acid sequence comparison placed the **catalytic domain** of CenD in family A, subtype 1, of β-1,4-glucanases. The repeated sequences are more than 40% identical to the sequences of three repeats in CenB and are related to the repeats of fibronectin type III. CenD hydrolyzed the β-1,4-glucosidic bond with retention of anomeric configuration. The activities of CenD towards various cellulosic substrates were quite different from those of CenA and CenB.

L21 ANSWER 57 OF 60 CAPLUS COPYRIGHT 2004 ACS on STN

1992:422311 Document No. 117:22311 The adsorption of a bacterial cellulase and its two isolated domains to crystalline cellulose. Gilkes, Neil R.; Jervis, Eric; Henrissat, Bernard; Tekant, Bahar; Miller, Robert C., Jr.; Warren, R. Antony J.; Kilburn, Douglas G. (Dep. Microbiol., Univ. British Columbia, Vancouver, BC, B6T 1Z3, Can.). Journal of Biological Chemistry, 267(10), 6743-9 (English) 1992. CODEN: JBCHA3. ISSN: 0021-9258.

AB CenA is a bacterial cellulase (β-1,4-glucanase) comprised of a globular **catalytic domain** joined to an extended **cellulose-binding domain** (CBD) by a short **linker** peptide. The adsorption of CenA and its two isolated domains to crystalline cellulose was analyzed. CenA and CBD·PTCenA (the CBD of Cellulomonas fimi endo-β-1,4-glucanase A, plus the 22 amino-proximal amino acids of the proline- and threonine-rich interdomain liner peptide) adsorbed rapidly to cellulose at 30°, and no net desorption of protein was observed during the following 16.7 h. There was no detectable adsorption of the **catalytic domain**. Scatchard plots of adsorption data for CenA and for CBD·PTCenA were nonlinear (concave upward). The adsorption of CenA and CBD·PTCenA exceeded 7 and 8 μmol/g cellulose, resp., but saturation was not attained at the highest total protein concns. employed. A new model for adsorption was developed to describe the interaction of a large ligand (protein) with a lattice of overlapping potential binding sites (cellobiose residues). A relative equilibrium association constant (Kr) of 40.5 and 45.3 L·g cellulose-1 was estimated for CenA and CBD·PTCenA, resp., according to this model. A similar Kr value (33.3 L·g-1) was also obtained for Cex, a C. fimi enzyme which contains a related CBD but which hydrolyzes both β1,4-xylosidic and β-1,4-glucosidic bonds. It was estimated that the CBD occupies approx. 39 cellobiose residues on the cellulose surface.

L21 ANSWER 58 OF 60 CAPLUS COPYRIGHT 2004 ACS on STN

1992:54482 Document No. 116:54482 Multiple domains in endoglucanase B (CenB) from Cellulomonas fimi: functions and relatedness to domains in other polypeptides. Meinke, A.; Gilkes, N. R.; Kilburn, D. G.; Miller, R. C., Jr.; Warren, R. A. J. (Dep. Microbiol., Univ. British Columbia, Vancouver, BC, V6T 1W6, Can.). Journal of Bacteriology, 173(22), 7126-35 (English) 1991. CODEN: JOBAAY. ISSN: 0021-9193.

AB Endoglucanase B (CenB) from the bacterium C. fimi is divided into five discrete domains by **linker** sequences rich in proline and hydroxyamino acids (Meinke, A., et al., 1991). The **catalytic domain** of 608 amino acids is at the N terminus. The sequence of the first 477 amino acids in the **catalytic domain** is related to the sequences of cellulases in family E, which includes prokaryotic and eukaryotic enzymes. The sequence of the last 131 amino acids of the **catalytic domain** is related to sequences present in a number of cellulases from different families. The **catalytic domain** alone can bind to cellulose, and this binding is mediated at least in part by the C-terminal 131 amino acids. Deletion of these 131 amino acids reduces but does not eliminate activity. The **catalytic domain** is followed by three domains which are repeats of a 98-amino-acid sequence. The repeats are .apprx.50% identical to two repeats of 95 amino acids in a chitinase from Bacillus circulans which are related to fibronectin type III repeats (Watanabe, T., et. al., 1990). The C-

terminal of 101 amino acids is related to sequences, present in a number of bacterial cellulases and xylanases from different families, which form **cellulose-binding domains** (CBDs). It functions as a CBD when fused to a heterogenous polypeptide. Cells of Escherichia coli expressing the wild-type cenB gene accumulate both native CenB and a stable proteolytic fragment of 41 kDa comprising the three repeats and the C-terminal CBD. The 41-kDa polypeptide binds to cellulose but lacks enzymic activity.

L21 ANSWER 59 OF 60 CAPLUS COPYRIGHT 2004 ACS on STN
1991:488241 Document No. 115:88241 Deletion of the **linker** connecting the catalytic and **cellulose-binding domains** of endoglucanase A (CenA) of Cellulomonas fimi alters its conformation and catalytic activity. Shen, Hua; Schmuck, Maria; Pilz, Ingrid; Gilkes, Neil R.; Kilburn, Douglas G.; Miller, Robert C., Jr.; Warren, R. Antony J. (Dep. Microbiol., Univ. British Columbia, Vancouver, BC, V6T 1W5, Can.). Journal of Biological Chemistry, 266(17), 11335-40 (English) 1991. CODEN: JBCHA3. ISSN: 0021-9258.

AB The Pro-Thr box is a **linker** of 23 amino acids ((PT)4T(PT)7) connecting the **catalytic domain** and the **cellulose-binding domain** (CBD) of endoglucanase A (CenA) from the bacterium C. fimi. Deletion of the Pro-Thr box alters the conformation of CenA by changing the relative orientation of the **catalytic domain** and the CBD. The tertiary structures of the **catalytic domain** and the CBD appear to be unchanged. The change in conformation reduces the catalytic efficiency of the enzyme and masks one of two protease-sensitive sites between the domains. The deletion does not affect the adsorption of the enzyme to microcryst. cellulose, but it does affect its desorption from cellulose. The results suggest that the Pro-Thr box in CenA has an extended, kicked, and rigid conformation.

L21 ANSWER 60 OF 60 CAPLUS COPYRIGHT 2004 ACS on STN
1991:442823 Document No. 115:42823 Unusual sequence organization in CenB, an inverting endoglucanase from Cellulomonas fimi. Meinke, A.; Braun, C.; Gilkes, N. R.; Kilburn, D. G.; Miller, R. C., Jr.; Warren, R. A. J. (Dep. Microbiol., Univ. British Columbia, Vancouver, BC, V6T 1W5, Can.). Journal of Bacteriology, 173(1), 308-14 (English) 1991. CODEN: JOBAAY. ISSN: 0021-9193.

AB The nucleotide sequence of the cenB gene was determined and used to deduce the amino acid sequence of endoglucanase B (CenB) of C. fimi. CenB comprises 1012 amino acids and has a mol. weight of 105,905. The polypeptide is divided by so-called **linker** sequences rich in proline and hydroxyamino acids into 5 domains: **catalytic domain** of 607 amino acids at the N terminus, followed by 3 repeats of 98 amino acids each which are >60% identical, and a C-terminal domain of 101 amino acids which is 50% identical to the **cellulose-binding domains** of C. fimi cellulases Cex and CenA. A deletion mutant of the cenB gene encodes a polypeptide lacking the C-terminal 333 amino acids of CenB. The truncated polypeptide is catalytically active and, like intact CenB, binds to cellulose, suggesting that CenB has a second cellulose-binding site. The sequence of amino acids 1 to 461 of CenB is 35% identical, with a further 15% similarity, to that of a cellulase from avocado, which places CenB in cellulase family E. CenB releases mostly cellobiose and cellotetraose from cellohexaose. Like CenA, CenB hydrolyzes the β-1,4-glucosidic bond with inversion of the anomeric configuration. The pH optimum for CenB is 8.5, and that for CenA is 7.5.

=> D L20 1-2 CBIB ABS

L20 ANSWER 1 OF 2 CAPLUS COPYRIGHT 2004 ACS on STN

2003:717659 Document No. 139:242281 Sequences of a **linker** region of Trichoderma reesei **cellobiohydrolase** I gene and use for improving thermostability. Adney, William S.; Decker, Stephen R.; Mccarter, Suzanne; Baker, John O.; Nieves, Rafael; Himmel, Michael E.; Vinzant, Todd B. (USA). U.S. Pat. Appl. Publ. US 2003170861 A1 20030911, 17 pp. (English). CODEN: USXXCO. APPLICATION: US 2002-31496 20020114.

AB The invention provides sequences of a liker region between a **catalytic domain** and a **cellulose binding domain** of a modified cellobiohydrolase. A nucleic acid mol. having a nucleic acid sequence that encodes a **linker** region of exoglucanase, said nucleic acid sequence comprising the nucleic sequence 5'-GGCGGAAACCCGCCTGGCACCACC-3'.

L20 ANSWER 2 OF 2 CAPLUS COPYRIGHT 2004 ACS on STN

2001:191006 Document No. 134:363254 Non-hydrolytic disruption of crystalline structure of cellulose by **cellulose binding domain** and **linker** sequence of **cellobiohydrolase** I from Penicillium janthinellum. Gao, Peiji; Chen, Guanjun; Wang, Tianhong; Zhang, Yingshu; Liu, Jie (State Key Laboratory of Microbial Technology, Shandong University, Jinan, 250100, Peop. Rep. China). Shengwu Huaxue Yu Shengwu Wuli Xuebao, 33(1), 13-18 (Chinese) 2001. CODEN: SHWPAU. ISSN: 0582-9879. Publisher: Shanghai Kexue Jishu Chubanshe.

AB The synergistic effects between cellobiohydrolase (CBHI) and endoglucanase (EG) on biodegrdn. of natural cellulose were studied. A recombinant plasmid (pUC18C), containing the gene fragment encoding **cellulose binding domain** (CBD) with its **linker** sequence of **cellobiohydrolase** I from P. janthinellum (CBDCBHI), was cloned from pUC18-181. The **catalytic domain** region of cbd I gene in plasmid pUC18C was deleted by in vitro DNA manipulations and then E. coli JM 109 was transformed for production of LacZ-CBD fusion protein. The active LacZ-CBD fusion protein was digested by papain and then purified by re-exclusion chromatog. The purified peptide sequence of CBDCBHI had the ability of binding crystalline cellulose. The morphol. and structural changes of cotton fibers after binding CBDCBHI were studied by SEM, calorimetry, and x-ray diffraction. CBDCBHI had a high binding capacity to cellulose and caused non-hydrolytic disruption of crystalline cellulose and then caused release of short fibers. IR spectroscopy and x-ray diffraction show that destabilization was caused by non-hydrolytic disruption of cellulose and disruption of hydrogen bonds in crystalline cellulose. The efficiency of crystalline cellulose degradation was enhanced by synergistic action of CBDCBHI with EGI. The results showed that the **cellulose-binding domain** with its **linker** may play an important role in crystalline cellulose degradation

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=> S L14(3A)L15
L23         406 L14(3A)L15

=> S L4 AND L23
L24           0 L4 AND L23

=> S L14 AND L15
L25        6914 L14 AND L15

=> S L25 AND L4
L26          11 L25 AND L4

=> D 1-11 CBIB ABS
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L26 ANSWER 1 OF 11 CAPLUS COPYRIGHT 2004 ACS on STN

2003:1007005 Document No. 140:55604 Improved recombinant production of aspartic protease in eukaryotic hosts by modifying **glycosylation** site. Van Den Brink, Johannes Maarten; Harboe, Marianne K.; Petersen, Steen Guldager; Rahbek-Nielsen, Henrik (Chr. Hansen A/S, Den.). PCT Int. Appl. WO 2003106484 A1 20031224, 54 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NI, NO, NZ, OM, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG, TR. (English). CODEN: PIXXD2. APPLICATION: WO 2003-DK398 20030616. PRIORITY: DK 2002-922 20020617.

AB The present invention provides a method to achieve an enhanced amount of secreted heterologous proteins, in particular aspartic proteases, from eukaryotic hosts by modifying a polypeptide coding for an aspartic protease modified so as to comprise at least one addnl. -N-X-T- **glycosylation** site. The present inventors have found that the recombinant production capacity of an aspartic protease, such as e.g. chymosin, can be increased by an alteration of a specific **glycosylation** site. More specifically, by incorporating a new N-X-T **glycosylation** site into a polypeptide comprising an aspartic protease **amino acid** sequence. In working example 1 herein a new N-X-T **glycosylation** site is incorporated into the active mature part of an aspartic protease and in working example 3 a new N-X-T **glycosylation** site is incorporated into a linker sequence situated upstream of a pro-fragment of an aspartic protease. In both cases the result was increased yields of the recombinantly produced aspartic protease without significantly reducing the activity of the enzyme as compared to its native counterpart. Method of producing bovine chymosin recombinantly in an Aspergillus host strain, expressed as fusion proteins with glucoamylase is described. In this example the prochymosin B gene sequence is replaced by a new, synthetic chymosin B gene that comprises one optimized N-**glycosylation** site.

L26 ANSWER 2 OF 11 CAPLUS COPYRIGHT 2004 ACS on STN

2003:856051 Document No. 139:349661 Production of functional antibodies in filamentous fungi for diagnosis and therapeutic uses. Power, Scott D.; Wang, Huaming; Ward, Michael (Genencor International, Inc., USA). PCT Int. Appl. WO 2003089614 A2 20031030, 78 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NI, NO, NZ, OM, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG, TR. (English). CODEN: PIXXD2. APPLICATION: WO 2003-US12246 20030417. PRIORITY: US 2002-PV373889 20020418; US 2002-PV411540 20020918; US 2002-PV411537 20020918; US 2003-PV452134 20030304.

AB Described herein are methods for the production of monoclonal antibodies in filamentous fungi host cells. The monoclonal antibodies are expressed as full-length fusion proteins that retain functional antigen binding and antibody-dependent cellular cytotoxicity capabilities. Improvements in the cleavage of the glucoamylase-light chain fusion protein to yield a mature antibody are also provided. The antibodies produced in filamentous fungi show equivalent pharmacokinetic disposition to antibodies produced in mammalian cells.

L26 ANSWER 3 OF 11 CAPLUS COPYRIGHT 2004 ACS on STN

2003:842062 Document No. 140:211660 Cloning of a gene encoding thermostable **cellobiohydrolase** from Thermoascus aurantiacus and its expression in yeast. Hong, J.; Tamaki, H.; Yamamoto, K.; Kumagai, H. (Graduate School of Biostudies, Division of Integrated Life Sciences, Kyoto University, Sakyo-ku, Kyoto, 606-8502, Japan). Applied Microbiology and Biotechnology, 63(1), 42-50 (English) 2003. CODEN: AMBIDG. ISSN: 0175-7598. Publisher: Springer-Verlag.

AB A gene encoding a **cellobiohydrolase** (CBH) was isolated from Thermoascus aurantiacus IFO 9748 and designated as cbh1. The deduced **amino acid** sequence encoded by cbh1 showed high homol. with the sequence of glycoside hydrolase family 7. To confirm the sequence of the gene encoding the CBH, the cloned gene was expressed in the yeast Saccharomyces cerevisiae, in which no cellulase activity was found, and the gene product was purified and subjected to enzymic characterization. The recombinant enzyme was confirmed as a CBH by anal. of the reaction product and designated as CBHI. Recombinant CBHI retained more than 80% of its initial activity after 1 h of incubation at 65 °C and was stable in the pH range 3.0-9.0. The optimal temperature for enzyme activity was about 65 °C and the optimal pH was about 6.0. The recombinant enzyme was found to be highly glycosylated and this **glycosylation** was shown to contribute to the thermostability of the enzyme. CBHI expression was shown to be induced at higher temperature in T. aurantiacus.

L26 ANSWER 4 OF 11 CAPLUS COPYRIGHT 2004 ACS on STN

2002:295763 Document No. 137:30394 Maturation of barley cysteine endopeptidase expressed in Trichoderma reesei is distorted by incomplete processing. Nykanen, Marko J.; Raudaskoski, Marjatta; Nevalainen, Helena; Mikkonen, Anita (Department of Biological and Environmental Science, University of Jyvaskyla, Jyvaskyla, 40351, Finland). Canadian Journal of Microbiology, 48(2), 138-150 (English) 2002. CODEN: CJMIAZ. ISSN: 0008-4166. Publisher: National Research Council of Canada.

AB Maturation of barley cysteine endopeptidase B (EPB) in Trichoderma reesei was studied with metabolic inhibitors, Western blotting, and immuno microscopy. The inactive 42-kDa recombinant EPB proprotein, first detected in apical cells, was sequentially processed in a time-dependent manner to a secreted polypeptide of 38.5 kDa, and thereafter, to polypeptides of 37.5, 35.5, and 32 kDa exhibiting enzyme activity both in the hyphae and culture medium. The sizes of the different forms of recombinant EPB were in accordance with mol. masses calculated from the deduced **amino acid** sequence, assuming cleavage at four putative Kex2p sites present in the 42-kDa proprotein. Both the liquid and the zymogram in-gel activity assays indicated that the 32-kDa enzyme produced in T. reesei in vivo was 2 kDa larger and four times less active than the endogenous EPB. Brefeldin A treatment prevented the last Kex2p processing step of EPB from a 35.5- to a 32-kDa protein. This coincided with a significant increase in the immuno-gold label for EPB and in modified Golgi-like bodies, which suggests that the processing step probably took place in medial Golgi. A 30.5-kDa EPB polypeptide was observed when **glycosylation** was inhibited by tunicamycin (TM) or when deglycosylation was carried out enzymically. Deglycosylation increased the enzyme activity twofold, which was also indicated by an increased fluorescence by TM treatment in the zymogram in-gel activity assay. Simultaneous incubation with TM and monensin produced a peptide of 31.5 kDa. Therefore, monensin may inhibit the final processing step of an unglycosylated EPB by an unknown protease in the fungus. In any case, the final recombinant EPB product in Trichoderma differs from the mature endogenous 30-kDa enzyme produced in barley.

L26 ANSWER 5 OF 11 CAPLUS COPYRIGHT 2004 ACS on STN

2001:683006 Document No. 136:380878 Cloning and sequence analysis of **cellobiohydrolase** II gene from Trichoderma Koningii K801. Zhu, Lingxiang; Yu, Wei; Liang, Gaiqin; Dong, Zhiyang (Institute of Microbiology, Chinese Academy of Sciences, Beijing, 100080, Peop. Rep. China). Junwu Xitong, 20(2), 174-177 (Chinese) 2001. CODEN: JUXIFB. ISSN: 1007-3515. Publisher: Kexue Chubanshe.

AB The **cellobiohydrolase** II gene, cbh2 was amplified by polymerase chain reaction (PCR) from an enhanced cellulase- producing strain, Trichoderma koningii K, and then was cloned to T- easy vector. Sequence comparisons showed that cbh2 of T.koningii was identical to that of T.reesei with the exception of 2 bp in the coding region, which caused an **amino acid** substituted in the mature CBHII protein. The similarity values between CBHII gene of T.koningii and reported cbh2 gene of Trichoderma reesei was 99.89%. The cbh2 gene of T.koningii was 1611 bp long, and the coding region was interrupted by three short introns. The derived CBHII protein sequence was 471 **amino acids** long and three putative N-**glycosylation** sites was found.

L26 ANSWER 6 OF 11 CAPLUS COPYRIGHT 2004 ACS on STN

2001:50790 Document No. 134:111240 Site-directed mutagenesis for the preparation of **cellobiohydrolase** reduced **glycosylation** variants CBHIN45A, CBHIN270A, and CBHIN384A and their use in ethanol production. Adney, William S.; Decker, Stephen R.; Lantz McCarter, Suzanne; Baker, John O.; Nieves, Rafael; Himmel, Michael E.; Vinzant, Todd B. (Midwest Research Institute, USA). PCT Int. Appl. WO 2001004284 A1 20010118, 21 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW; RW: AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE. (English). CODEN: PIXXD2. APPLICATION: WO 2000-US19007 20000713. PRIORITY: US 1999-PV143711 19990713.

AB The invention provides a method for enhancing the catalytic activity of exoglucanase **cellobiohydrolase** cloned from in Trichoderma reesei, which can be used for ethanol production from pretreated biomass such as acid hydrolyzed hardwoods. Three reduced **glycosylation** variant CBHIN45A, CBHIN270A, and CBHIN384A are generated by site-directed mutagenesis to change asparagine to alanine at the positions of 45, 270, and 384 of **cellobiohydrolase**. The invention also relates to constructing expression vector to produce recombinant enzyme variants and their industrial preparation using Aspergillus awamori cells.

L26 ANSWER 7 OF 11 CAPLUS COPYRIGHT 2004 ACS on STN

1998:26796 Document No. 128:189839 **Cellobiohydrolase** I from Trichoderma reesei: identification of an active site nucleophile and additional information on sequence including the **glycosylation** pattern of the core protein. Klarskov, Klaus; Piens, Kathleen; Stahlberg, Jerry; Hoj, Peter B.; Van Beeumen, Jozef; Claeyssens, Marc (Department of Biochemistry, Physiology and Microbiology, University of Gent, Ghent, B-9000, Belg.). Carbohydrate Research, 304(2), 143-154 (English) 1997. CODEN: CRBRAT. ISSN: 0008-6215. Publisher: Elsevier Science Ltd..

AB (R,S)-3,4-Epoxybutyl β-cellobioside, but not the corresponding Pr and pentyl derivs., inactivates specifically and irreversibly **cellobiohydrolase** I from Trichoderma reesei by covalent modification of Glu212, the putative active-site nucleophile. The position and identity of the modified **amino acid** residue were determined using a combination of comparative liquid chromatog. coupled online to electrospray ionization mass spectrometry, tandem mass spectrometry and microsequencing. It was found that the core protein

corresponds to the N-terminal sequence pyrGlu1-Gly434(Gly435) of intact **cellobiohydrolase** I. In the particular enzyme samples investigated, the asparagine residues in positions 45, 270 and 384 are each linked to a single 2-acetamido-2-deoxy-D-glucopyranose residue.

L26 ANSWER 8 OF 11 CAPLUS COPYRIGHT 2004 ACS on STN
1997:682830 Document No. 127:328325 A comparative structural characterization of two **cellobiohydrolases** from Trichoderma reesei: a high resolution electron microscopy study. Lee, H. Jong; Brown, R. Malcolm, Jr. (Dep. Botany, Univ. Texas, Austin, TX, 78713, USA). Journal of Biotechnology, 57(1-3), 127-136 (English) 1997. CODEN: JBITD4. ISSN: 0168-1656. Publisher: Elsevier.

AB The mol. structure of 2 homogeneously purified **cellobiohydrolases** from T. reesei was studied employing high resolution transmission electron microscopy (TEM) and digital image processing. By combining low-dose TEM, a 3% solution of methylamine tungstate as the neg. staining agent, and digital image processing, it was possible to view intact mol. architectures of **cellobiohydrolases** from T. reesei. **Cellobiohydrolase** I (CBH I) and **cellobiohydrolase** II (CBH II) had ellipsoidal heads and long, extended helical tails. CBH I was composed of a head with an average diameter of 4.5 nm. The average length of CBH I was 15.1 nm. CBH II's head had an average diameter of 4.4 nm. The average length of CBH I was 13.4 nm. Overall, the structure of the digitally enhanced TEM images of these 2 glycosyl hydrolases correlated reasonably well with the reported models based on small angle x-ray scattering (SAXS), x-ray crystallog., and NMR imaging studies, thereby establishing the reliability of high-resolution TEM studies in a novel way. The linker regions (LRs) of the 2 CBHs were visualized. The LRs of both enzymes are probably helical in nature, suggesting an extended peptide sequence that lacks secondary structure due to the absence of **amino acid- amino acid** interactions in this region. A possible structural and functional role of O-**glycosylation** affiliated with these linker regions was discussed on the basis of combining the TEM observations with known biochem. data.

L26 ANSWER 9 OF 11 CAPLUS COPYRIGHT 2004 ACS on STN
1987:114581 Document No. 106:114581 Cloning, characterization, and expression in Saccharomyces cerevisiae of endoglucanase I from Trichoderma reesei. Van Arsdell, Janelle N.; Kwok, Shirley; Schweickart, Vicki L.; Ladner, Martha B.; Gelfand, David H.; Innis, Michael A. (Dep. Microb. Genet., Cetus Corp., Emeryville, CA, 94608, USA). Bio/Technology, 5(1), 60-4 (English) 1987. CODEN: BTCHDA. ISSN: 0733-222X.

AB The cloning, partial characterization, and expression in yeast of the endo-β-glucanase [9074-99-1] isoenzyme I (EGI) gene from Trichoderma reesei is reported. DNA sequencing revealed significant homol. at the **amino acid** level between EGI and exocellobiohydrolase [**37329-65-0**] isoenzyme I (CBHI), but there are differences in codon utilization at homologous **amino acids** and in the intron/exon structure. These possibly reflect a mechanism for preventing recombination between closely related genes of the cellulase family. The coding sequence for the mature protein with its signal peptide was inserted into an expression plasmid containing yeast transcription control sequences. Yeast colonies transformed with this plasmid secrete enzymically active, hyperglycosylated EGI to the culture medium. This novel **glycosylation** appears to render the enzyme more resistant to thermal inactivation.

L26 ANSWER 10 OF 11 CAPLUS COPYRIGHT 2004 ACS on STN
1987:62062 Document No. 106:62062 Homology between cellulase genes of Trichoderma reesei: complete nucleotide sequence of the endoglucanase I gene. Penttila, Merja; Lehtovaara, Paivi; Nevalainen, Helena; Bhikhabhai,

Ramagauri; Knowles, Jonathan (Biotech. Lab., VTT, Espoo, SF-02150, Finland). Gene, 45(3), 253-63 (English) 1986. CODEN: GENED6. ISSN: 0378-1119.

AB The filamentous fungus T. reesei produces several endoglucanase I (EG) [9074-99-1] and **cellobiohydrolases** (CBH) [**37329-65-0**] which are involved in cellulose hydrolysis in a complex synergistic manner. The gene and the full-length cDNA coding for the major endoglucanase EG-I were cloned, sequenced, and compared to the cbh1 gene sequence to clarify the relationship between the EG and CBH classes of cellulases. The deduced 437-**amino acid** (aa)-long EG-I protein with a 22-aa-long signal peptide is 45% identical in aa sequence with CBH-I. The best conserved region is found at the C terminus and shows about 70% homol. The data suggest that the 2 enzymes have arisen from a common ancestor by gene duplication. Despite this, the intron positions have not been conserved in these genes which both contain 2 short introns. The deduced EG-I sequence contains 6 putative N- **glycosylation** sites, and a putative O-glycosylated region is found near the C terminus, closely resembling a similar region at the C terminus of CBH-I. Comparison of the aa sequences suggests that the evolutionary divergence of EG-I from CBH-I has involved 4 sep. 10-20 aa deletions from the ancestral protein.

L26 ANSWER 11 OF 11 CAPLUS COPYRIGHT 2004 ACS on STN

1986:162949 Document No. 104:162949 Cellulase system of Trichoderma reesei: trichoderma strain improvement and expression of Trichoderma cellulases in yeast. Shoemaker, Sharon P. (Cetus Corp., USA). World Biotech Rep., Volume 2, 593-600. Online Publ.: Pinner, UK. (English) 1984. CODEN: 54WPAA.

AB The genes for endoglucanase E.C. 3.2.1.4 (E6I) [9012-54-8] and **cellobiohydrolase** E.C. 3.2.1.91 (CBHI) [**37329-65-0**] of Trichoderma reesei were cloned into yeast, after deleting the introns and placing the coding sequences of the genes under the control of a yeast promoter and downstream regulatory sequences. The N-terminal **amino acid** sequence of the recombinant CBHI was identical to that of wild type, indicating that the secretory leader peptide was correctly processed. However, the yeast CBHI was extensively glycosylated, in contrast to the slightly glycosylated native CBHI. The specific activities of both the cloned and native enzymes were approx. equal. The yeast EGI also had a higher mol. weight than did native EGI, suggesting it was also more glycosylated. Trichoderma Strain improvement is reviewed.

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=> E ADNEY W/AU
=> S E4-E6
             8 "ADNEY W S"/AU
             1 "ADNEY WILLIAM"/AU
            55 "ADNEY WILLIAM S"/AU
L27         64 ("ADNEY W S"/AU OR "ADNEY WILLIAM"/AU OR "ADNEY WILLIAM S"/AU)

=> E DECKER STEPHEN/AU
=> S E2-E11
             6 "DECKER STEPHAN"/AU
             2 "DECKER STEPHEN"/AU
            18 "DECKER STEPHEN A"/AU
             1 "DECKER STEPHEN ANDREW"/AU
            21 "DECKER STEPHEN R"/AU
             1 "DECKER STEPHEN ROBERT"/AU
             1 "DECKER STEPHEN W"/AU
             1 "DECKER STEVE"/AU
             1 "DECKER STEVE R"/AU
             1 "DECKER STEVEN"/AU
L28         53 ("DECKER STEPHAN"/AU OR "DECKER STEPHEN"/AU OR "DECKER STEPHEN
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                A"/AU OR "DECKER STEPHEN ANDREW"/AU OR "DECKER STEPHEN R"/AU OR
                "DECKER STEPHEN ROBERT"/AU OR "DECKER STEPHEN W"/AU OR "DECKER
                STEVE"/AU OR "DECKER STEVE R"/AU OR "DECKER STEVEN"/AU)

=> E MCCARTER S/AU
=> S E8-E10
             2 "MCCARTER SUZANNE"/AU
             2 "MCCARTER SUZANNE L"/AU
             1 "MCCARTER SUZANNE LANTZ"/AU
L29          5 ("MCCARTER SUZANNE"/AU OR "MCCARTER SUZANNE L"/AU OR "MCCARTER
               SUZANNE LANTZ"/AU)

=> E BAKER JOHN/AU
=> S E3-E58
            62 "BAKER JOHN"/AU
            23 "BAKER JOHN A"/AU
             1 "BAKER JOHN A G"/AU
             1 "BAKER JOHN A JR"/AU
             3 "BAKER JOHN ALBERT"/AU
             1 "BAKER JOHN ALLEN"/AU
             1 "BAKER JOHN ASHLEY"/AU
             6 "BAKER JOHN B"/AU
            51 "BAKER JOHN C"/AU
             1 "BAKER JOHN C JR"/AU
            18 "BAKER JOHN D"/AU
             3 "BAKER JOHN D JR"/AU
            10 "BAKER JOHN DAVID"/AU
             1 "BAKER JOHN DAYTON JR"/AU
            37 "BAKER JOHN E"/AU
             2 "BAKER JOHN EDWARD"/AU
             7 "BAKER JOHN F"/AU
            22 "BAKER JOHN G"/AU
             2 "BAKER JOHN GEOFFREY"/AU
             1 "BAKER JOHN GERARD"/AU
            17 "BAKER JOHN H"/AU
             1 "BAKER JOHN HARDIN JR"/AU
             1 "BAKER JOHN HARRIS EDWARD II"/AU
             7 "BAKER JOHN HOWARD"/AU
            11 "BAKER JOHN I"/AU
             1 "BAKER JOHN ISAAC"/AU
             6 "BAKER JOHN J"/AU
             1 "BAKER JOHN JAMES"/AU
            71 "BAKER JOHN K"/AU
             1 "BAKER JOHN KEITH"/AU
             1 "BAKER JOHN KENNETH"/AU
             4 "BAKER JOHN L"/AU
             1 "BAKER JOHN L IV"/AU
            24 "BAKER JOHN M"/AU
             1 "BAKER JOHN M JR"/AU
             3 "BAKER JOHN MARSHALL"/AU
            23 "BAKER JOHN MICHAEL"/AU
             3 "BAKER JOHN MILTON JR"/AU
             1 "BAKER JOHN N"/AU
            41 "BAKER JOHN O"/AU
             1 "BAKER JOHN OLEN"/AU
            13 "BAKER JOHN P"/AU
             1 "BAKER JOHN PATTON"/AU
             3 "BAKER JOHN PERRY"/AU
           115 "BAKER JOHN R"/AU
             3 "BAKER JOHN R J"/AU
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              1 "BAKER JOHN RAYMOND"/AU
             11 "BAKER JOHN RICHARD"/AU
              1 "BAKER JOHN ROWLAND"/AU
              6 "BAKER JOHN S"/AU
              3 "BAKER JOHN T"/AU
             56 "BAKER JOHN W"/AU
              1 "BAKER JOHN WAINWRIGHT"/AU
              3 "BAKER JOHN WELLSTOOD"/AU
              1 "BAKER JOHN WILLIAM"/AU
             15 "BAKER JOHN WM"/AU
L30         706 ("BAKER JOHN"/AU OR "BAKER JOHN A"/AU OR "BAKER JOHN A G"/AU OR
                "BAKER JOHN A JR"/AU OR "BAKER JOHN ALBERT"/AU OR "BAKER JOHN
                ALLEN"/AU OR "BAKER JOHN ASHLEY"/AU OR "BAKER JOHN B"/AU OR
                "BAKER JOHN C"/AU OR "BAKER JOHN C JR"/AU OR "BAKER JOHN D"/AU
                OR "BAKER JOHN D JR"/AU OR "BAKER JOHN DAVID"/AU OR "BAKER JOHN
                DAYTON JR"/AU OR "BAKER JOHN E"/AU OR "BAKER JOHN EDWARD"/AU OR
                "BAKER JOHN F"/AU OR "BAKER JOHN G"/AU OR "BAKER JOHN GEOFFREY"/
                AU OR "BAKER JOHN GERARD"/AU OR "BAKER JOHN H"/AU OR "BAKER
                JOHN HARDIN JR"/AU OR "BAKER JOHN HARRIS EDWARD II"/AU OR "BAKER
                 JOHN HOWARD"/AU OR "BAKER JOHN I"/AU OR "BAKER JOHN ISAAC"/AU
                OR "BAKER JOHN J"/AU OR "BAKER JOHN JAMES"/AU OR "BAKER JOHN
                K"/AU OR "BAKER JOHN KEITH"/AU OR "BAKER JOHN KENNETH"/AU OR
                "BAKER JOHN L"/AU OR "BAKER JOHN L IV"/AU OR "BAKER JOHN M"/AU
                OR "BAKER JOHN M JR"/AU OR "BAKER JOHN MARSHALL"/AU OR "BAKER
                JOHN MICHAEL"/AU OR "BAKER JOHN MILTON JR"/AU OR "BAKER JOHN
                N"/AU OR "BAKER JOHN O"/AU OR "BAKER JOHN OLEN"/AU OR

=> E NIEVES R/AU
=> S E3-E8
              3 "NIEVES R"/AU
              5 "NIEVES R A"/AU
              2 "NIEVES RAFAEL"/AU
             13 "NIEVES RAFAEL A"/AU
              1 "NIEVES RAFAEL ANGEL"/AU
              1 "NIEVES RAFAEL E"/AU
L31          25 ("NIEVES R"/AU OR "NIEVES R A"/AU OR "NIEVES RAFAEL"/AU OR "NIEV
                ES RAFAEL A"/AU OR "NIEVES RAFAEL ANGEL"/AU OR "NIEVES RAFAEL
                E"/AU)

=> E HIMMEL M/AU
=> S E3-E7
             10 "HIMMEL M"/AU
             33 "HIMMEL M E"/AU
              4 "HIMMEL MICHAEL"/AU
            109 "HIMMEL MICHAEL E"/AU
              1 "HIMMEL MICHAEL EDWARD"/AU
L32         157 ("HIMMEL M"/AU OR "HIMMEL M E"/AU OR "HIMMEL MICHAEL"/AU OR
                "HIMMEL MICHAEL E"/AU OR "HIMMEL MICHAEL EDWARD"/AU)

=> E VINZANT T/AU
=> S E4,E4
              3 "VINZANT T B"/AU
              3 "VINZANT T B"/AU
L33           3 ("VINZANT T B"/AU OR "VINZANT T B"/AU)

=> S L27,L28,L29,L30,L31,L32,L33
L34         865 (L27 OR L28 OR L29 OR L30 OR L31 OR L32 OR L33)

=> S L34 AND L2
L35          16 L34 AND L2
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=> S L35 NOT (L20,L22)
L36 15 L35 NOT ((L20 OR L22))

=> D 1-15 CBIB ABS

L36 ANSWER 1 OF 15 CAPLUS COPYRIGHT 2004 ACS on STN
2003:830940 Document No. 140:76005 Heterologous expression of Trichoderma reesei 1,4-β-D-glucan **cellobiohydrolase** (Cel 7A). **Adney, William S.**; Chou, Yat-Chen; **Decker, Stephen R.**; Ding, Shi-You; **Baker, John O.**; Kunkel, Glenna; Vinzant, Todd B.; **Himmel, Michael E.** (Biotechnology Division for Fuels and Chemicals, National Renewable Energy Laboratory, National Bioenergy Center, Golden, CO, 80401, USA). ACS Symposium Series, 855(Applications of Enzymes to Lignocellulosics), 403-437 (English) 2003. CODEN: ACSMC8. ISSN: 0097-6156. Publisher: American Chemical Society.

AB **Cellobiohydrolase** I (Cel7A) from Trichoderma reesei is generally recognized as being the most important enzyme in the construction of engineered component cellulase systems designed for hydrolysis of microcryst. cellulose. We previously reported that full-length T. reesei Cel7A can be expressed from E. coli or P. pastoris, however the enzyme was either produced as insol. inclusion bodies or hyperglycosylated resp. In this study, we report the expression of active and stable full length Cel7A from transformed A. awamori and insect cells, and addnl. attempts to produce active soluble enzyme in E. coli. From this and previous work, we have concluded that E. coli and P. pastoris are unsuitable for expression of full length or the catalytic domain of Cel7A for the purpose of conducting site-directed-mutagenesis. We compare the activity kinetics and thermal denaturation properties of two forms of recombinant and the wild type T. reesei Cel7A.

L36 ANSWER 2 OF 15 CAPLUS COPYRIGHT 2004 ACS on STN
2003:179512 Heterologous expression, purification, and characterization of the glycosyl hydrolase family 7 **cellobiohydrolases** from Trichoderma reesei and Penicillium funiculosum. **Adney, William S.**; Chou, Yat-Chen; **Decker, Stephen R.**; **Baker, John O.**; **Himmel, Michael E.** (National Bioenergy Center, National Renewable Energy Laboratory, Golden, CO, 80401, USA). Abstracts of Papers, 225th ACS National Meeting, New Orleans, LA, United States, March 23-27, 2003, CELL-006. American Chemical Society: Washington, D. C. (English) 2003. CODEN: 69DSA4.

AB Many members of the phylum Dikaryomycota (both Ascomycetes and Basidiomycetes) are known for their role in the biodegrdn. and recycling of organic matter in nature. Their ability to digest cellulosic biomass (e.g. leaf litter and wood), and in the case of many Basidiomycetes both cellulose and lignin, is of great interest to the emerging bioenergy industry, since biomass represents an enormous renewable resource for the production of fuels and chems. Among the notable genera of industrially important fungi that produce GH family 7 **cellobiohydrolases** are Trichoderma and Penicillium. The **cellobiohydrolases** from this structural family are generally recognized as being the most important enzymes in the construction of engineered component cellulase systems designed for hydrolysis of microcryst. cellulose. In this study, we report the heterologous expression of active and stable full-length GH family 7 **cellobiohydrolases** from transformed Aspergillus awamori. We compare the kinetics and biochem. properties of the recombinant forms to the wild type enzymes and examine the role of posttranslational modifications on protein structure and activity.

L36 ANSWER 3 OF 15 CAPLUS COPYRIGHT 2004 ACS on STN

2001:344101 Document No. 135:88924 Fingerprinting Trichoderma reesei hydrolases in a commercial cellulase preparation. **Vinzant, T. B.** ; **Adney, W. S.**; Decker, S. R.; Baker, J. O.; Kinter, M. T.; Sherman, N. E.; Fox, J. W.; **Himmel, M. E.** (Biotechnology Center for Fuels and Chemicals, National Renewable Energy Laboratory, Golden, CO, 80401, USA). Applied Biochemistry and Biotechnology, 91-93(Symposium on Biotechnology for Fuels and Chemicals, 2000), 99-107 (English) 2001. CODEN: ABIBDL. ISSN: 0273-2289. Publisher: Humana Press Inc..

AB Polysaccharide-degrading enzymes from com. T. reesei broth were subjected to "fingerprint" anal. by high-resolution 2-dimensional gel electrophoresis. Forty-five spots from 11 + 25 cm Pharmacia gels were analyzed by LC-MS/MS and the resulting peptide sequences were compared to existing databases. Understanding the roles and relations of component enzymes from the T. reesei cellulase system acting on complex substrates is key to the development of efficient artificial cellulase systems for the conversion of lignocellulosic biomass to sugars. These studies suggest follow-on work comparing induced and non-induced T. reesei cells at the proteome level, which may elucidate substrate-specific gene regulation and response.

L36 ANSWER 4 OF 15 CAPLUS COPYRIGHT 2004 ACS on STN

2001:50790 Document No. 134:111240 Site-directed mutagenesis for the preparation of **cellobiohydrolase** reduced glycosylation variants CBHIN45A, CBHIN270A, and CBHIN384A and their use in ethanol production. **Adney, William S.**; **Decker, Stephen R.**; Lantz McCarter, Suzanne; **Baker, John O.**; **Nieves, Rafael**; **Himmel, Michael E.**; Vinzant, Todd B. (Midwest Research Institute, USA). PCT Int. Appl. WO 2001004284 A1 20010118, 21 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW; RW: AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE. (English). CODEN: PIXXD2. APPLICATION: WO 2000-US19007 20000713. PRIORITY: US 1999-PV143711 19990713.

AB The invention provides a method for enhancing the catalytic activity of exoglucanase **cellobiohydrolase** cloned from in Trichoderma reesei, which can be used for ethanol production from pretreated biomass such as acid hydrolyzed hardwoods. Three reduced glycosylation variant CBHIN45A, CBHIN270A, and CBHIN384A are generated by site-directed mutagenesis to change asparagine to alanine at the positions of 45, 270, and 384 of **cellobiohydrolase**. The invention also relates to constructing expression vector to produce recombinant enzyme variants and their industrial preparation using Aspergillus awamori cells.

L36 ANSWER 5 OF 15 CAPLUS COPYRIGHT 2004 ACS on STN

1999:585199 Document No. 131:319411 Cloning and Expression of Trichoderma reesei **Cellobiohydrolase** I in Pichia pastoris. Godbole, Shubhada; **Decker, Stephen R.**; **Nieves, Rafael A.**; **Adney, William S.**; Vinzant, Todd B.; **Baker, John O.**; Thomas, Steven R.; **Himmel, Michael E.** (Biotechnology Center for Fuels and Chemicals, National Renewable Energy Laboratory, Golden, CO, 80401, USA). Biotechnology Progress, 15(5), 828-833 (English) 1999. CODEN: BIPRET. ISSN: 8756-7938. Publisher: American Chemical Society.

AB Pichia pastoris was transformed with the Trichoderma reesei cbh1 gene, and the recombinant enzyme was purified and analyzed kinetically and by CD. The P. pastoris rCBH I was recognized by MoAb raised to T. reesei CBH I but was found in multiple mol. weight species on SDS-PAGE gels. Carbohydrate content

determination and SDS-PAGE western anal. indicated that the recombinant protein was hyperglycosylated, although a species very similar in mol. weight to the T. reesei enzyme could be isolated chromatog. The P. pastoris rCBH I also demonstrated activity toward soluble and insol. substrates (i.e., pNPL and Sigmacell), although at a level significantly lower than the wild-type enzyme. More seriously, the yeast-expressed enzyme showed non-wild-type secondary structure by CD. We conclude that P. pastoris may not serve as an adequate host for the site-directed mutagenesis of T. reesei CBH I.

L36 ANSWER 6 OF 15 CAPLUS COPYRIGHT 2004 ACS on STN
1996:324812 Document No. 125:27152 Cloning and expression of full-length Trichoderma reesei **cellobiohydrolase** I cDNAs in Escherichia coli. Laymon, Robert A.; **Adney, William S.**; Mohagheghi, Ali; **Himmel, Michael E.**; Thomas, Steven R. (Appl. Biol. Sci. Branch, Natl. Renewable Energy Lab., Golden, CO, 80401, USA). Applied Biochemistry and Biotechnology, 57/58(Seventeenth Symposium on Biotechnology for Fuels and Chemicals, 1995), 389-397 (English) 1996. CODEN: ABIBDL. ISSN: 0273-2289. Publisher: Humana.

AB The process of converting lignocellulosic biomass to ethanol via fermentation depends on developing economic sources of cellulases. Trichoderma reesei **cellobiohydrolase** (CBH) I is a key enzyme in the fungal cellulase system; however, specific process application requirements make modification of the enzyme by site-directed mutagenesis (SDM) an attractive goal. To undertake SDM investigations, an efficient, cellulase-free host is required. To test the potential of Escherichia coli as a host, T. reesei CBH I cDNA was expressed in E. coli strain GI 724 as a C-terminal fusion to thermostable thioredoxin protein. Full-length expression of CBH I was subsequently verified by mol. weight, Western blot anal., and activity on soluble substrates.

L36 ANSWER 7 OF 15 CAPLUS COPYRIGHT 2004 ACS on STN
1995:452114 Document No. 122:208675 Enzymically active conjugates and fusion proteins of **cellobiohydrolase** and β-glucosidase for saccharification of cellulose. **Baker, John O.; Himmel, Michael E.**; Grohmann, Karel; Thomas, Steven R. (Midwest Research Institute, USA). PCT Int. Appl. WO 9429460 A1 19941222, 27 pp. DESIGNATED STATES: W: FI; RW: AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE. (English). CODEN: PIXXD2. APPLICATION: WO 1994-US6528 19940610. PRIORITY: US 1993-74373 19930611.

AB Enzymically active heterodimers of β-glucosidase and **cellobiohydrolase** are prepared by crosslinking with a bifunctional reagent or as fusion products. The ability of the β-glucosidase to catalyze hydrolysis of cellobiose to glucose, and the ability of **cellobiohydrolase** to bind to crystalline cellulose and to catalyze the cleavage of cellobiosyl residues from the non-reducing ends of the cellulose chains, are all retained in the combined mol. The crosslinking of monomeric Aspergillus niger β-glucosidase by crosslinking with glutaraldehyde and reduction of the Schiff base with NaBH4 to obtain a dimer with normal kinetics is demonstrated.

L36 ANSWER 8 OF 15 CAPLUS COPYRIGHT 2004 ACS on STN
1995:225676 Document No. 122:181432 Components of Trichoderma reesei cellulase complex on crystalline cellulose. Three-dimensional visualization with colloidal gold. **Nieves, Rafael A.**; Todd, Roberta J.; Ellis, Robert P.; **Himmel, Michael E.** (Applied Biol. Sci. Branch, Natl. Renewable Energy Lab., Golden, CO, 80401-3393, USA). ACS Symposium Series, 566(Enzymatic Conversion of Biomass for Fuels Production), 236-43 (English) 1994. CODEN: ACSMC8. ISSN: 0097-6156.

Publisher: American Chemical Society.

AB A review with 9 refs. Bacterial cellulose and pretreated natural aspen cellulose were used as substrates for observation of cellulose-bound cellulases. Specific monoclonal antibodies which had previously been adsorbed to 10-nm and 15-nm gold spheres were used to detect bound endoglucanase and **cellobiohydrolase** via transmission electron microscopy. Three-dimensional electron micrographs demonstrated individually bound cellulases as well as clusters of bound enzymes. Significant changes in the interpretations of the micrographs were seen when these were observed in a three-dimensional format as opposed to a two-dimensional view. The three-dimensional electron micrographs indicated the sensitivity of this technique for these studies by revealing individual enzymes bound to individual cellulose microfibril(s).

L36 ANSWER 9 OF 15 CAPLUS COPYRIGHT 2004 ACS on STN

1994:321433 Document No. 120:321433 A new thermostable endoglucanase, Acidothermus cellulolyticus E1. Synergism with Trichoderma reesei CBH I and comparison to Thermomonospora fusca E5. **Baker, John O.**; **Adney, William S.**; **Nieves, Rafael A.**; Thomas, Steven R.; Wilson, David B.; **Himmel, Michael E.** (Alternat. Fuels Div., Natl. Renewable Energy Lab., Golden, CO, 80401, USA). Applied Biochemistry and Biotechnology, 45-46, 245-56 (English) 1994. CODEN: ABIBDL. ISSN: 0273-2289.

AB A new thermostable endoglucanase, Acidothermus cellulolyticus E1, and another bacterial endoglucanase, E5 from Thermomonospora fusca, each exhibit striking synergism with a fungal **cellobiohydrolase** (Trichoderma reesei CBH I) in the saccharification of microcryst. cellulose. In either case did the ratio of endoglucanase to exoglucanase that demonstrated maximum synergism coincide exactly with the ratio that actually released the maximum quantity of soluble sugar for a given total cellulase loading. The difference between the two ratios, after significant hydrolysis of the substrate, was considerably larger in the case of A. cellulolyticus E1. For both endoglucanase pairings with CBH I, the offset between the ratio for maximum synergism and the ratio of maximal soluble sugar production was found to be a function of digestion time.

L36 ANSWER 10 OF 15 CAPLUS COPYRIGHT 2004 ACS on STN

1993:97058 Document No. 118:97058 Thermal and pH stress in thermal denaturation of Trichoderma reesei **cellobiohydrolase** I. Supporting evidence for a two-transition model. **Baker, John O.**; **Himmel, Michael E.** (Alternative Fuels Div., Natl. Renewable Energy Lab., Golden, CO, 80401, USA). ACS Symposium Series, 516(Biocatalyst Design for Stability and Specificity), 83-101 (English) 1993. CODEN: ACSMC8. ISSN: 0097-6156.

AB The structure and thermal denaturation of Trichoderma reesei **cellobiohydrolase** I (CBH I) have been investigated using fluorescence, chemical modification, and differential scanning calorimetry (DSC) techniques. The results of both fluorescence quenching with cesium ion and chemical modification with N-bromosuccinimide indicate that at least seven, and possibly eight, of the nine tryptophan residues in the CBH I catalytic core region are in exposed positions at or near the surface of the native mol. A biphasic perturbation of the CBH I intrinsic fluorescence reveals that the CBH I core region is capable of binding more than one mol. of cellobiose and suggests that this addnl. bound mol. may be important for the stabilization of the core region against thermal denaturation. When the temperature of a solution of CBH I (pH 7.5 in 50 mM phosphate) is ramped through its denaturation zone (approx. 28° - 48° at this pH), a sharp, sigmoidal change, centered at approx. 36°, is observed in the polarization of the tryptophan fluorescence of the protein. This polarization change precedes both the endothermic peak maximum (40.15°) observed in DSC under the same conditions and the second (40.3°) and larger of

two component peaks involved to explain the asym. shape of the DSC peak. The midpoint of the fluorescence polarization change is much more closely correlated with the first (37.2°) and smaller of the deconvoluted component peaks. The fluorescence-polarization data thus provide supporting evidence for the component transitions, the existence of which has heretofore rested only on math. inference, and thereby for the two-transition model proposed earlier for the denaturation.

L36 ANSWER 11 OF 15 CAPLUS COPYRIGHT 2004 ACS on STN
1992:587207 Document No. 117:187207 Thermal denaturation of Trichoderma reesei cellulases studied by differential scanning calorimetry and tryptophan fluorescence. Baker, J. O.; Tatsumoto, K.; Grohmann, K.; Woodward, J.; Wichert, J. M.; Shoemaker, S. P.; **Himmel, M. E.** (Fuels Chem. Res. Eng. Div., Sol. Energy Res. Inst., Golden, CO, 80401, USA). Applied Biochemistry and Biotechnology, 34-35, 217-31 (English) 1992. CODEN: ABIBDL. ISSN: 0273-2289.

AB The thermal denaturation of 4 purified T. reesei cellulase components, **cellobiohydrolase** (CBH) I, CBH II, endoglucanase (EG) I, and EG II, has been monitored using a combination of classical temperature/activity profiles, DSC, and thermal scanning fluorescence emission spectrometry. Significant correlations were found between the results of enzyme activity studies and the results obtained through the more direct phys. approaches, in that both DSC and the activity studies showed EG II (Tm = 75°) to be much more thermostable (by 10-11°) than the other 3 enzymes, all 3 of which were shown by both activity profiles and DSC to be very similar in thermal stability. The temperature dependence of the wavelength of maximum tryptophan emission showed a parallel result, with the 3 enzymes exhibiting less thermostable activity being grouped together in this regard, and EG II differing from the other three in maintaining a less-exposed tryptophan microenvironment at temps. as high as 73°. The DSC results suggested that at least 2 transitions are involved in the unfolding of each of the cellulase components, the first (lower-temperature) of which may be the one correlated with activity loss.

L36 ANSWER 12 OF 15 CAPLUS COPYRIGHT 2004 ACS on STN
1991:674080 Document No. 115:274080 Visualization of Trichoderma reesei **cellobiohydrolase** I and endoglucanase I on aspen cellulose by using monoclonal antibody-colloidal gold conjugates. **Nieves, Rafael A.**; Ellis, Robert P.; Todd, Roberta J.; Johnson, Timothy J. A.; Grohmann, Karel; **Himmel, Michael E.** (Biotechnol. Res. Branch, Sol. Energy Res. Inst., Golden, CO, 80401, USA). Applied and Environmental Microbiology, 57(11), 3163-70 (English) 1991. CODEN: AEMIDF. ISSN: 0099-2240.

AB Monoclonal antibodies (MAbs) specific for **cellobiohydrolase** I (CBH I) and endoglucanase I (EG I) were conjugated to 10- and 15-nm colloidal gold particles, resp. The binding of CBH I and EG I was visualized by utilizing the MAb-colloidal gold probes. The visualization procedure involved immobilization of cellulose microfibrils on copper electron microscopy grids, incubation of the cellulose-coated grids with cellulase(s), binding of MAb-colloidal gold conjugates to cellulase(s), and visualization via transmission electron microscopy. CBH I was seen bound to apparent crystalline cellulose as well as apparent amorphous cellulose. EG I was seen bound extensively to apparent amorphous cellulose with minimal binding to crystalline cellulose.

L36 ANSWER 13 OF 15 CAPLUS COPYRIGHT 2004 ACS on STN
1991:467460 Document No. 115:67460 Thermal unfolding of Trichoderma reesei CBH I. Baker, J. O.; Mitchell, D. J.; Grohmann, K.; **Himmel, Michael E.** (Solar Fuels Res. Div., Solar Energy Res. Inst., Golden, CO,

80401, USA). ACS Symposium Series, 460(Enzymes Biomass Convers.), 313-30 (English) 1991. CODEN: ACSMC8. ISSN: 0097-6156.

AB Purified **cellobiohydrolase** I (CBH I) was subjected to thermal denaturation anal. using differential scanning calorimetry (DSC). As many as 3 endothermic structural transitions were detected during heating of the native enzyme from 20° to 75° at a range of pH values 4.80-8.34. DSC anal. of the separated proteolytic fragments representing the catalytic core and cellulose-binding tail regions of the CBH I mol. revealed that all 3 of the transitions arose from the core region. Two of these transitions were inferred as the deconvoluted constituent peaks of an asym. peak that appeared at 64° at pH 4.8 but was shifted to 33.4° as the pH was increased to 8.34. A much smaller 3rd transition at 55° appeared to be much less pH-dependent, but was observed only when the 2 major transitions were shifted to temps. substantially lower than 55° (as at pH 7.5 or above). Cellobiose at 100 mM dramatically stabilized the CBH I mol., shifting the position of the major, 2-component peak from 64° to 72° at pH 4.8, and from 33.4° to 51.9° at pH 8.34.

L36 ANSWER 14 OF 15 CAPLUS COPYRIGHT 2004 ACS on STN

1990:419914 Document No. 113:19914 Monoclonal antibody purification of Trichoderma reesei EG I. **Nieves, Rafael A.**; Ellis, Robert P.; **Himmel, Michael E.** (Dep. Microbiol., Colorado State Univ., Fort Collins, CO, 80523, USA). Applied Biochemistry and Biotechnology, 24-25, 397-406 (English) 1990. CODEN: ABIBDL. ISSN: 0273-2289.

AB The purification of T. reesei endoglucanase I (cellulase; EC 3.2.1.4) (I) by immunoaffinity chromatog. is presented. Monoclonal antibodies (MAb) specific for I were prepared and characterized. Chromatog. of a com. cellulose preparation on Affigel-10-immobilized anti-I MAb yielded a preparation containing .apprx.400 μg of protein and contaminated by **cellobiohydrolase** I (II). This preparation was concentrated and applied to an affinity column containing anti-II MAb. The void volume obtained from this column contained 20-50 μg purified I.

L36 ANSWER 15 OF 15 CAPLUS COPYRIGHT 2004 ACS on STN

1990:233684 Document No. 112:233684 Cross-reactive and specific monoclonal antibodies against **cellobiohydrolases** I and II and endoglucanases I and II of Trichoderma reesei. **Nieves, R. A.**; **Himmel, M. E.**; Todd, R. J.; Ellis, R. P. (Dep. Microbiol., Colorado State Univ., Fort Collins, CO, 80523, USA). Applied and Environmental Microbiology, 56(4), 1103-8 (English) 1990. CODEN: AEMIDF. ISSN: 0099-2240.

AB Splenocytes derived from mice inoculated with a com. cellulase preparation or purified cellulases were fused with a stable myeloma cell line (SP2/0). Specific monoclonal antibodies to **cellobiohydrolases** I and II and endoglucanases I and II were established. In addition to specific monoclonal antibodies, stable hybridoma cell lines were established which produced monoclonal antibodies that recognized similar epitopes possessed by ≥2 of the above cellulases. By obtaining monospecific antibodies for all 4 individual cellulases, the role and function of the individual cellulases can thus be studied in greater detail.